CORT

The Structure of some Frog Trematodes

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THE STRUCTURE OF SOME FROG TREMATODES

BY

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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY

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ENTITLED The Structure of Some Frog Trematodes

BE ACCEPTED AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE

DEGREE OF Master of Arts

Henry B. Ward In Charge of Major Work

Henry B. Ward Head of Department

Recommendation concurred in:

Committee
on
Final Examination

INTRODUCTION

Since February 1910, when I first became interested in frog parasites I have made collections from various sources. In this material I have found several new species, and can report the finding of several others probably for the first time since their original descriptions. As the original descriptions are incomplete, I have been able to add some new facts and correct some errors. In the present paper I will make no attempt to exhaust the subject either for any region or for any genus, but will give the descriptions of the different forms found, as far as my material and the time at my disposal have allowed me to carry the work. The first part of the paper will cover the general parasitic infection of the hosts examined, the second part will be taken up with a careful consideration of the lung and bladder flukes, and the last part will comprise the facts I have been able to learn in regard to the other trematodes found.

My material comes from four principal sources; first from frogs obtained from Spung of North Judson, Indiana; second from frogs caught in the vicinity of Urbana, Ill.; third from frogs and toads collected around Havana, Ill.; and fourth from frogs coming from the "Bait and Angler's Co.," Chicago, which were given me by the Department of Physiology. Also I have had the use of material from Dr. Henry B. Ward's private collection, and of material obtained by Dr. Ward from Canada thru the kindness of Dr. R. R. Ben-

sley. Prof. Pratt of Haverford College, Penn. sent a slide of Ostiolum formosum Pratt which was very valuable for comparison, and the specimens of one species of lung fluke, which came from an Oklahoma toad were given me by Hermann Douthitt. I wish to express my gratitude to Dr. Henry B. Ward for the material he has put at my disposal, and for the aid and advice he has given me in the course of my work, and also to George La Rue for the help he has given me in technique.

My records of parasitic examinations are incomplete in some particulars. The frogs obtained last spring from Spung, were examined merely for lung flukes, and part of the frogs from the Physiology department this spring, were given me in such condition and such numbers, that they were examined only for bladder flukes. Below I give my records for the frogs and toads examined for this work.

Lot number and location	Date	Hosts examined	Hosts infected	Organs infected	Name of hosts
Lot I. Spung frogs No. Judson, Ind.	Feb. Apr.	58	36	Lungs	R. pipiens
Lot II Calhoun, Ill.	Apr.	16	None		R. areolata
Lot III Crystal Lake, Urbana, Ill.	May	18	None		R. pipiens
Lot IV Crystal Lake, Urbana, Ill.	June	3	1	Bladder	R. pipiens
Lot V Crystal Lake, Urbana, Ill.	June	1	1	Lungs	R. virescens
Lot VI Havana, Ill.	June July	25	None		Common toad
Lot VII Crystal Lake, Urbana, Ill.	Sept. Oct.	19	4	Bladder Rectum	R. pipiens
Lot VIII Crystal Lake, Urbana, Ill.	Sept. Oct.	5	2	Bladder	R. virescens
Lot IX Bait & Angler Co., Chicago, Ill.	Feb. 1911	15	9 10 3	Lungs Bladder Rectum & con. tissue	R. pipiens
Lot X Bait & Angler Co., Chicago, Ill. Physiology Dept.	Feb. 1911	15	11	Bladder	R. pipiens

Names and number of trematodes found.

Haematoloechus medioplexus	H. similiplexus	H. longiplexus	Diplodiscus	Gorgodera sp.	Gorgoderina attenuata	Gorgoderina Sp. I.	Gorgoderina Sp. II.	Clinostomum larvae	Remarks
198	10	12							Only lungs examined
						2			
		4							
			8			5			
				51					
29	6	5	4		12		9	6	
					16		8		Examined only for bladder flukes.

From my examinations as shown by the above table I have gathered the following species of trematodes, of the genus Haematoloechus, H. medioplexus, H. similiplexus, H. longiplexus and H. breviplexus; of the genus Gorgodera, a new species; of Gorgoderina, Gn. attenuata, and two undescribed species; and finally a species of Diplodiscus and a number of Clinostomum larvae. My examinations have dealt almost exclusively with Rana pipiens. Since most of my material came from frogs shipped in from Spung of North Judson, Indiana, and from the Bait and Angler Co. of Chicago, being therefore of doubtful origin, not much data of importance in regard to trematode distribution can be obtained from the above table. Perhaps the only points of value to be noted are the scarcity of trematode infection in the frogs about Urbana, Ill., and the frogs and toads in the region of Havana, Ill. as compared with the Indiana frogs and those obtained from the "Bait and Angler Co." of Chicago, which probably came from regions further north.

The technique used with all the different species was fairly uniform. The worms were usually killed after shaking by Looss' method (Looss, 1901) in 5% corrosive sublimate + 1% acetic acid. It was found best to transfer the specimens of Haematoloechus to distilled water before killing, in order that a part of the eggs might be evacuated. After this treatment they were killed without shaking. All specimens were left in the cold killing fluid from 10 - 24 hours. For staining in toto Mayer's Paracarmine, and Mayer's Haemalum or Delafield's Haematoxylin gave the best results. The worms were left over night in a concentrated Paracarmine stain

in 70% alcohol, and then destained rapidly and thoroughly in 4 or 5% HCl in 70% alcohol. Some of my prettiest and most valuable preparations were made by this method. For the haematoxylin stains two or three days in a dilute stain, and then a rapid and rather complete destaining as above gave good results. For sections haematoxylin and acid fuschin or eosin for counterstains were my favorites. Much time was saved by staining in toto with Ehrlich's acid haematoxylin, differentiating on the slide after sectioning and then counterstaining in the higher grades of alcohol. By the use of this method it was made unnecessary to run the sections thru the lower grades of alcohol. The technique of both the lung and bladder flukes is rather difficult on account of the great mass of eggs present. Great care was necessary not to overheat the material in the slightest degree, and still to give it sufficient infiltration. All species as far as possible were studied alive, but practically all measurements were made from mounted material.

In taking up the detailed study of my material on frog trematodes I will deal first with the genus Haematoloechus.

The genus Haematoloechus. Looss, 1899.

Syn. Pneumonoeces. Looss, 1902.

The genus Haematoloechus - the common lung flukes of frogs and toads -, which at present writing contains ten species, was originally represented by the old form Distomum variegatum Rudolphi. Under this name these lung flukes were referred to from time to time from Rudolphi until 1899. The last and best description of D. variegatum is that of Looss.(Looss, 1894) Even

at this time Looss began to notice differences, and he figures two forms of this type showing distinct variations (Looss, 1894, taf. II, fig. 43 & 45). In 1899 (Looss, 1899: 600 -601) the same author following the policy he then adopted of splitting up the genus Distomum, established for this type of frog lung fluke the new genus Haematoloechus. At the same time he re-examined his old D. variegatum material, and succeeded in dividing it into three distinct species. The largest of these he designated as the type species of the genus Haematoloechus under the name of H. variegatus. The other two he named H. similis and H. asper. In naming H. similis Looss makes the following statement, (Looss, 1899: 602)

"Diese deart charakterisirte Form ist bereits auf meinen alten Präparaten mit dem Namen Distomum simile beseichnet, und ich scheide sie hiermit als selbständige Species Haematoloechus similis positiv von H. variegatus ab." Stiles later (Stiles, 1902: 20) calls attention to the fact that Looss in mentioning the name D. simile in connection with his preparations had given this species two names D. simile and H. similis. The former of these names is what Stiles calls a still born homonym. He therefore proposes as a substitute for Looss' name the new combination Haematoloechus similigenus. I believe that Stiles' new name should be accepted. Looss names (Looss, 1899: 602) his third species Haematoloechus asper, and because he has only two specimens labels it as a species inquirenda.

In 1902 (Looss, 1902; 732), on account of Stäls' hemipteron genus Haematoloecha established in 1874, Looss changed the generic name Haematoloechus to Pneumonoeces. He did this in-

fluenced by the argument of Braun (Braun, 1901: 55) that if family or sub-family names were formed from generic names which differed only in ending they would be identical.

The question of whether the same word used in different genders, and differing only in ending, can be used for different genera, under the law of homonyms, is a matter of much dispute. I am inclined to agree with Stiles' interpretation of the rule of homonyms in this respect. He writes: (Stiles, 1901: 173) "As for masculine, feminine and neuter names, I fail to find any justification for rejecting one in case another already exists." On this basis Stiles accepts as valid the two names Tetracotylus Monticelli 1892 and Tetracotyle Filippi 1854. If we accept this view, we must reject the name Pneumonoeces, and go back to the original name Haematoloechus proposed by Looss in 1899 for this genus.

In 1902 Stafford (Staf., 1902: 895-912) under the title of "American representatives of Distomum variegatum," described five new species of this genus from Canada naming them H. longiplexus, H. breviplexus, H. varioplexus, H. similiplexus and H. medioplexus. Pratt before Stafford's paper was in print (Pratt, 1903: 34-37) described a frog lung fluke under the name Ostiolum formosum nov. gen. nov. sp. Stafford in 1905 (Staf., 1905: 687) accepted Looss' change in generic name and renames his species giving them the generic name Pneumonoeces. He writes:

"In the same issue of the Zool. Jahrb. (Heft. 3-6 1902: 895-912) in which I described these species, Looss (p.732) substituted the generic name Pneumonoeces for Haematoloechus. Ostiolum formosum Pratt (Mark Ann. vol. 1903) is doubtless the same worm as

Pn. medioplexus." While I cannot agree with Stafford in accepting Looss' generic name Pneumonoeces as a substitute for Haematoloechus, my investigations have convinced me that the form described by Pratt as O. formosum is the same as Stafford's species H. medioplexus, that this form belongs to the genus Haematoloechus and that Pratt's new genus and species cannot stand. My reasons for holding this view will be given later in the present paper.

Besides Looss' original three species and Stafford's five Canadian species two additional species have since been described, one as Pn. capyristes from a frog in India by Klein (Klein, 1905: 60-64) and the other as Pn. complexus from Rana pipiens in North Carolina by Seely (Seely, 1906: 248-252). Klein and Seely have both accepted without question Looss' change in the name of this genus. According to my opinion, as stated above, Klein's species should be known as Haematoloechus capyristes, and Seely's as Haematoloechus complexus. At the present writing then ten species are ascribed to the genus Haematoloechus Looss, 1899.

From the parasitic examinations recorded above, I have obtained specimens of H.medioplexus, H. similiplexus, H. longiplexus, and H.breviplexus. I have a great abundance of material of the first of these species,a much less amount for the second two,and but one toto preparation of the fourth. The comparison of specimens of these four species with Stafford's descriptions,and Pratt's description of O.formosum, and a toto mount of O.formosum, have yielded some interesting conclusions,some new facts,and have made it possible to correct some errors in the original descriptions. In order to facilitate comparison and indicate relationships, I have included in ad-

dition to the discussion of points of interest in these four species, a brief specific diagnosis and synonymy of all the species of this genus. The type species H. variegatus will be the first to be considered.

1. Haematoloechus variegatus (Rud.,1819) Looss, 1899,

1819: Distomum variegatum Rud.,1819: 99, 378-9.
* 1894: Distomum variegatum Looss, 1894, taf. II. fig. 45 (in part) : 71.
1902: Pneumonoeces variegatus Looss, 1902: 806.

Specific diagnosis

Large forms 16-18 mm. in length, with the anterior end strikingly narrower than the posterior.

Cuticula smooth.

Oral sucker but slightly larger than the acetabulum.

Testes and ovary rather elongated and slightly irregular in contour.

Yolk glands extending along almost the entire length of the body.

Median portion of the uterus comparatively straight, and lateral folds extending up to the ovary.

Eggs light brown in color. Length, maximum 0.032 mm., minimum 0.025 mm., and average 0.029 mm. Width maximum 0.0189 mm., minimum 0.0126 mm., and average 0.015 mm.

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* For fuller synonomy of D. variegatum see Looss, 1894: 71.

2. Haematoloechus similigenus. Stiles, 1902: 20.

1894: Distomum variegatum. Looss, 1894: taf. II. fig. 43. (In part.) 71.

1899: Haematoloechus similis. Looss, 1899: 602.

1902: Pneumonoeces similis. Looss, 1902: 806, 762.

Specific diagnosis,

Medium sized 7-10 mm. in length by about 2 mm. in width, in which the anterior end shows very little diminution in size.

Cuticula with spines.

Oral sucker but slightly larger than the acetabulum.

Testes round or slightly elongated.

Ovary round or oval.

Yolk glands short not extending into the posterior extremity of the body.

Median portion of the uterus not greatly folded, the lateral longitudinal folds extending forward to the anterior testis.

Eggs dark brown. Length max. 0.042 mm., min. 0.0336 mm., av. 0.0378 mm. Width max. 0.021 mm., min. 0.0168 mm., av. 0.019 mm.

3. Haematoloechus asper sp. inq. Looss, 1899: 603.

1894: Distomum variegatum. Looss, 1894: 71 (In part)

1905: Pneumonoeces asper. (Looss, 1902: 732) Klein, 1905: 64.

Specific diagnosis

Size and form of body agree with H. similigenus.

Cuticle all over the body drawn up into little wart like

points.

Oral sucker but slightly larger than the acetabulum.

Yolk glands reaching into the extremity of the body.

Lateral longitudinal folds of the uterus present.

Eggs 0.055 mm. x 0.029 mm.

The diagnoses of Looss' species are of necessity very short, and some important points are missing. The description of Distomum variegatum by Looss (Looss, 1894: 71-82) is based upon 19 specimens of this type. When he divided this species and established the new genus Haematoloechus (Looss, 1899: 601-602) he restudied this material and sorted it into the three distinct species. Of these nineteen specimens,10 were named H. similis, 7 H. variegatus, and 2 H. asper sp. inq. This separation is made in a foot note to the diagnosis of the genus Haematoloechus (Looss, 1899: 601-603). In this note also he calls attention to the fact that one of the drawings given for Distomum variegatum in 1894 (Looss, 1894: taf. II. fig. 43) is of a specimen which belongs in the H. similis group, and that another (Looss, 1894: taf. II. fig. 45) is of H. variegatus. Of H. asper sp. inq., which is founded from two specimens, there is no published drawing. In his description of D. variegatum Looss gives the following hosts (Looss, 1894: 72) - Rana esculenta, Bombinator igneus and Bufo cinereus. From this description there is no way of telling which of the three separated species are present in each of these hosts.

4. Haematoloechus longiplexus. Stafford, 1902: 901.

1905: Pneumonoeces longiplexus. Stafford, 1905: 687.

1905: Pneumonoeces longiplexus. Klein, 1905: 64.

Specific diagnosis

Occurs in Rana pipiens, R. virescens and R. catesbiana.

Rather wide and thick, varying in length ffom 3 mm. up to 15 mm. with the thickness over half the width.

Whole surface covered with a dense coating of spines.

Oral sucker about twice the size of the acetabulum.

Pharynx about half the size of the oral sucker.

Testes much elongated, not lobed, back of the middle of the body and parallel for most of their length.

Ovary elongated, irregularly but not deeply lobed.

Yolk glands consisting of 5 - 12 groups of follicles on a side, with 15 - 25 follicles in a group, extending from just back of the oesophagus to the posterior extremity of the body.

Uterus voluminous rather simply coiled, with the longitudinal lateral folds extending up to the region of the pharynx. Direction of folds mostly longitudinal.

Eggs light brown in color, regularly oval, and measuring between 0.021 mm. and 0.024 mm. in length and from 0.014 mm. to 0.017 mm. in width.

Haematoloechus longiplexus is the first species of this genus described by Stafford (Stafford, 1902: 901 - 903). As I can find no mention of this form having been reported since its original description and as I am able to add some new facts, I shall take it up in some detail. In all the specimens of Rana pipiens, I have examined, I have found this form to the number of fifteen specimens in ten hosts. Besides these I have found four

individuals in a green frog - *R. virescens* - from the Crystal lake region near Urbana, Ill. These four are the only lung flukes that I have ever found around the University of Illinois. Stafford reports *H. longiplexus* as the commonest lung trematode of *Rana catesbiana* Shaw (the Bull-frog) in Ontario, Quebec, New Brunswick and Nova Scotia. Of the amount of infection he writes:

> "It does not occur in every frog of the above species but one can scarcely ever open two or three without finding it, and often to the number of ½ a dozen in each lung."

Stafford notes no peculiarity in the position of *H. longiplexus* in the lung. The specimens in *Rana pipiens* were found coiled up under the outer coating of the lung, forming small protuberances visible externally. They did not seem to be fastened by the suckers, for as soon as the lung was opened under saline solution they floated away without the tearing of the lung tissue necessary in loosening the other species, and straightened themselves out. When loose in the saline solution, they show considerable power of movement of the anterior end. In this position the worm is bluntly rounded and widest near the posterior end, while the anterior region can be protruded into a comparatively long slender neck, capable of considerable movement (Figure VII). All my mounted specimens are in the form of an elongated elipse, with the anterior end bluntly narrowed. (Fig. I.)

In size my forms vary from 3 - 7 mm. in length and from 1.2 mm. - 2.6 mm. in breadth. They have the greatest thickness in proportion to their width of any of the species of *Haematoloechus* which I have studied, a cross section thru the posterior part of the seminal receptacle measuring 1.3 mm. in width by 0.6 mm. in

thickness. (Fig. I a, 7.) All of my specimens of this species are smaller than the average of Stafford's forms from the bull-frog.

In my specimens of H. longiplexus the cuticula is 0.008 mm. to 0.01 mm. thick and is covered in all regions of the body with a very dense coating of spines about 0.001 mm. to 0.002 mm. apart and not over 0.003 mm. to 0.004 mm. long. The spines are set pointing slightly backward and reach only part way thru the cuticula. The cuticula sloughs off very easily, and in my mounts was only in places intact. My specimens show a marked variation from Stafford's account for this species. He writes:

"The cuticule in this species is thick and perfectly smooth, there being no trace of spines, in either the fresh worm or preserved specimens."

The oral sucker (Fig. I) in an average sized animal is round and measures about 0.33 mm. in diameter being a little over twice the size of the ventral sucker. This latter structure is a little over 1/3 of the distance from the anterior to the posterior end and measures 0.14 mm. in diameter and 0.07 mm. in depth. (Fig. I a, 5)

The digestive system offers few points of value in specific diagnosis. The pharynx (Fig. I) in a worm 7 mm. long is about ½ the width of the oral sucker, - oral sucker 0.36 mm. in length by 0.42 mm. in width, and the pharynx 0.18 mm. in width. In this same specimen the oesophagus (Fig. I) was about 0.25 mm. in length. As in all the other species of Haematoloechus the intestinal caeca (Fig. I & Fig. I a, 2-10) extend into the extreme posterior end of the body. Their width depends on the amount of food

material present. In one of my preparations they were so distended with disintegrating blood corpuscles, that in the region just back of the oesophagus their width practically equaled the width of the body of the animal. The suckers and digestive system in my forms agree closely with Stafford's description.

The excretory system (Fig. I a, 1-10) in H. longiplexus on account of differences in arrangement and size of the reproductive organs shows some variation from the other species. From the excretory pore at the middle of the dorsal posterior extremity of the animal, the large median vesicle runs forward along the dorsal median line below the posterior follicles of the yolk gland and then passes between the testes a little dorsal to the middle of the body. It holds about this same position until it reaches the posterior end of the seminal receptacle. Here it divides into two crura, which run slightly ventrally between the outer edge of the seminal receptacle and the inner border of the intestinal caeca up to the anterior end of this organ. From here they take their position ventral to the intestinal caeca and extend forward up to the anterior end of the pharynx. These crura extend much farther forward than in H. medioplexus.

The reproductive system (Fig. I, I a, I b, I c.) offers some very interesting points of difference from the other species of this genus. In Stafford's account, this system is not very thoroughly worked out, and there are no measurements.

The testes in H. longiplexus (Fig. I) are probably the most characteristic organs of the whole animal. They are much elongated entirely in the posterior half of the body and extend

almost to the ends of the intestinal caeca, with the one on the same side as the ovary slightly posterior and longer than the other. They are irregular cylinders tapering to both ends and longer than wide. At the anterior end they taper for about 2/5 of their length up to the point from which the vasa efferentia pass forward, and at the posterior end they are more bluntly pointed. In a mount of this species 4.4 mm. long, the posterior testis extended posteriorad 0.4 mm. farther than the anterior, within 0.54 mm. of the posterior end of the animal. The posterior testis of this same specimen was 1.26 mm. long by 0.32 mm. wide, and the anterior was 1.08 mm. long by 0.27 mm. wide. In another form 6.7 mm. long (Fig. I), the posterior testis was 1.6 mm. long by 0.53 mm. wide and the anterior was 1.4 mm. long by 0.49 mm. wide. In a cross section, 0.9 mm. wide by 0.54 mm. thick, (Fig. I a, 9.) thru about the middle region of the testes on another animal, the posterior testis measured 0.23 mm. in width by 0.43 mm. in thickness and the anterior 0.20 mm. in width by 0.36 mm. in thickness.

The vasa efferentia pass from the anterior ends of the testes forward to each side of the seminal vesicle and ovary very near to the dorsal body wall of the animal. Near the anterior end of the ovary they run ventralward, and unite into the vas deferens, which functions for almost its entire length as the seminal vesicle. The seminal vesicle at its posterior end is about 0.07 mm. wide, and is enclosed in the cirrus sac which in this region is in close contact with its walls. From this slightly dorsal position it winds forward and toward the ventral surface to the region about 0.36 mm. from the genital pore, where it narrows to the ductus

ejaculatorius (Fig. I c.). The cirrus sac at this region is very wide, and the space between it and the ductus ejaculatorius contains unicellular glandular cells, the prostate glands. In front of the ductus ejaculatorius is the cirrus (Fig. I c.), a thick-walled pear-shaped organ, which at its anterior end becomes narrow, and opens to the exterior along with the vagina at the common genital pore. The cirrus in H. longiplexus differs from that of H. medioplexus, (Fig. IV c) and H. variegatus (Looss, 1894, taf. VII, Fig. 134.) in being a larger thicker organ. The size and relationship of this organ have not been worked out for the other species of the genus. In one of my toto preparations I found a rather remarkable condition. The cavity of the cirrus was filled with ripe eggs. This shows a continuity of passage between the vagina and cirrus. As this was one of the specimens which had been placed in distilled water, I would be inclined to attribute the condition noted above to the reaction of the worm to an unnatural environment. This case is parallel to one which Looss notes for Distomum cygnoides (Looss, 1894: 61. taf. VI. Fig. 127).

Stafford makes no note of the male reproductive organs in this species beyond a brief description of the testes.

The ovary (Fig. I) in H. longiplexus is a somewhat oblong body, irregularly but not deeply lobed, lying a little to one side of the longitudinal axis of the worm, with its anterior end dorsal to the acetabulum. Sexual amphitypy is present for out of four toto preparations examined three had the ovary to the left and one to the right. In a preparation 6.8 mm. long (Fig. I) the ovary was situated 2.2 mm. from the anterior end with its long axis slightly diagonal to the long axis of the animal, and measured 0.85

mm. long by 0.72 mm. wide. In H. longiplexus the ovary is not nearly as thick as in the other species examined, in which it fills dorso - ventrally almost the entire thickness of the body of the animal. In a cross section thru its middle measuring 1.3 mm. wide by 0.63 mm. deep, (Fig. I a, 6) the ovary measured 0.65 mm. in width by 0.22 mm. in thickness.

Lying a little behind and ventral to the ovary at almost the exact center of the body of the animal is the receptaculum seminis, a regularly oval organ, slightly elongated with its long axis coinciding with the long axis of the worm. It is slightly larger than the ovary and fills the space between it and the ventral body wall overlapping it for about $\frac{3}{4}$ of its length. In a frontal section thru the middle of a specimen 3.6 mm. in length it measured 0.51 mm. in length by 0.38 mm. in width, and in a transverse section 0.98 mm. in width by 0.37 mm. in thickness it measured 0.32 mm. in width by 0.27 mm. in thickness. (Fig. I a, 7.)

The so-called shell gland (Fig. I b.) is a very extensive group of glandular cells, surrounding the oviduct and oötype. It lies dorsal and a little to one side of the ovary and just above the anterior 2/3 of the receptaculum seminis.

The oviduct (Fig. I b.) is a short narrow duct passing into the "shell gland" from the dorsal inside margin of the ovary just back of its middle. Almost immediately it receives the duct of the seminal (Fig. I b.) receptacle which is very short and passes upward from the dorsal side of that organ. Just after being joined by this duct the oviduct is met by the median duct of the yolk glands, and passes on thru the shell gland as the oötype,

which is recognizable by its heavy wall. The beginning of the uterus runs forward along the inside of the ovary. The relation of the ducts described above is typical for the genus Haematoloechus, the only differences among the species so far as they have been worked out, being occasioned by differences in the relative size and position of the organs. In H. longiplexus for example the organs are more bunched together than in H. medioplexus. (Fig. IV b.)

The yolk glands consist on each side of from 5 - 12 groups of from 15 - 25 follicles in a group, which extend along each side of the animal from just back of the oesophagus clear to the posterior extremity of the intestinal caeca. (Fig. I). They are dorsal in position and median groups are present both at the anterior and posterior ends. The individual follicles are small, measuring from about 0.07 mm. to o.11 mm. in length. The groups are connected by longitudinal ducts which are gathered from each side just back of the ovary into two median ducts. (Fig. I b) Just behind the shell gland they join into the median yolk reservoir, which passes into the oviduct, just after it has received the duct from the seminal receptacle.

The uterus (Fig. I, Fig. I a, 1-10) passes forward from the oötype around the anterior end of the ovary and then thru the region back of this organ in a series of transverse ventral folds. From just in front of the testes, it passes directly back to the posterior end of the animal, where it makes two very long and voluminous longitudinal folds outside the intestinal caeca up to the region of the pharynx. From the posterior end of the animal it

then passes forward in a series of simple, median coils ventral in position, and for the most part transverse in direction. (Fig. I) Toward its anterior end it narrows into a vagina about 0.45 mm. in length. (Fig. I c.)

The uterus in H. longiplexus is characterized by its large caliber - about 0.25 mm. in diameter, the simpleness of its coiling, the great length of the lateral longitudinal folds and the general longitudinal direction of its coils.

The eggs (Fig. VIII) are rather small for the genus, light brown in color, regularly oval in outline, disc shaped and measure from 0.021 mm. - 0.024 mm. in length by 0.014 - 0.017 mm. in width. (Fig. VIII.)

The above description supplements Stafford's original account for this species. (Staf. 1902: 901-903) My forms seem to be in general smaller than the ones Stafford studied from the bull-frog, and are covered by a thick coating of short spines. Since Stafford claims to have found "no trace of spines, in either the fresh worm or preserved sections," it would seem that the presence of spines is a variable factor and we would expect spined, spineless and partially spined varieties in the same species.

5. Haematoloechus breviplexus. Stafford, 1902: 904.
1905: Pneumonoeces breviplexus. Stafford, 1905: 687.
1905: Pneumonoeces breviplexus. Klein, 1905: 64.

Specific diagnosis

Hosts. Rana catesbiana Shaw, R. virescens Kalm and Bufo lentiginosus americanus.

Rather large spindle shaped worms measuring when mounted about 10 mm. in length by 2.5 mm. in width.

Cuticula thick, either smooth or with numerous backward projecting spines.

Oral sucker twice as large as the acetabulum.

Testes very large, irregular and bluntly lobed on both sides.

Ovary a very irregular and deeply lobed organ lying dorsal and to one side of the seminal receptacle.

Yolk glands composed of about ten groups of 12 - 20 follicles on a side and extending into the posterior end of the animal.

Lateral longitudinal folds of the uterus are variable, extending either up to the posterior region of the posterior testis or farther forward to the anterior region of this organ.

Eggs the smallest of the genus, measuring in length from 0.018 mm. - 0.021 mm. and in width from 0.013 mm. - 0.0156 mm.

I have one specimen of this species which is mounted as a toto preparation. (Fig. II.) As it comes from a different host, and a different region from Stafford's specimens, and as it shows several distinct variations from Stafford's description (Staf. 1902: 904-905) I shall describe it in as much detail as possible under the circumstances.

While my specimen of H. breviplexus shows several differences from Stafford's description of this species, there are present certain characteristics which make it unmistakable. My specimen is evidently somewhat contracted, and the uterus is

greatly distended with eggs so that where the coils are close together many parts are obscured. It is spindle-shaped tapering rather sharply to the posterior end, and having the anterior region somewhat more elongated. It is widest about the middle of the body in the region of the anterior testis, and measures 2.74 mm. in width by 9.4 mm. in length.

The cuticula in this specimen is sloughed off from most of the surface of the body, but here and there are patches which appear to be intact. These places show a thickness of 0.025 - 0.03 mm. or about three times that of H. longiplexus, and there is no trace of spines. This differs from Stafford's description. He writes: (Staf. 1902: 904)

"The cuticle in this species is thick but unlike the preceding species - H. longiplexus -, it is beset with numerous backwardly-projecting spines."

Here again the presence of spines seems to be a variable factor.

The oral sucker (Fig. II.) is subterminal measuring about 0.54 mm. in diameter. The acetabulum is located 3.5 mm. from the anterior end ventral to the receptaculum seminis and measures 0.25 mm. in diameter making its ratio to the oral sucker a little less than 1 - 2. Stafford notes this same ratio for his specimens but a smaller size for the acetabulum, i.e., 0.16 mm. in length by 0.12 mm. in depth in a sagittal section measuring 9 mm. in length by 0.8 in depth.

The pharynx which is the only part of the digestive system which can be made out in my mount is about 1/3 the size of the oral sucker, measuring 0.22 mm. in length by 0.18 mm. in width.

No part of the excretory system can be seen.

The testes (Fig. II.) are very large irregular bodies bluntly lobed on both sides lying diagonally the one behind the other, the posterior being on the same side as the ovary. They overlap for the distance of about 1 mm. and extend from within 4 mm. of the anterior end to within 2 mm. of the posterior end. The posterior is the largest, measuring 2.31 mm. in length by 1.09 mm. in width, while the anterior measures 1.71 mm. in length by 1.03 mm. in width. The size, shape and position of the testes described above is unique for the genus Haematoloechus and corresponds to Stafford's description for H. breviplexus.

The cirrus sac and the other structures of the anterior end are entirely hidden in my specimen by the great mass of eggs in the anterior folds of the uterus.

The ovary (Fig. II.) is a very deeply lobed irregular organ, lying dorsal and to the left side of the seminal receptacle. It has the general form of an isosceles triangle with the base less deeply lobed than the sides, and toward the inside. The length of this organ is 1.57 mm. and its width 0.92 mm. The above description corresponds to Stafford's account of the ovary in his specimens of this species.

The seminal receptacle (Fig. II.) lies just dorsal to the acetabulum, and is about the size of the ovary. Its exact outlines are obscured in my mount by the folds of the uterus.

The yolk glands extend along each side of the body from just back of the genital pore to about 1 mm. from the posterior end. They are dorsal to the other organs, with a posterior group of

follicles in the middle of the animal between the ends of the intestinal caeca. There are eight groups of follicles on the left side and ten on the right with from 12 - 20 follicles in a group, ranging in size from 0.057 mm. - 0.15 mm. and in shape from round or oval to pyriform.

In my specimen the anterior end is contracted, and a great mass of uterine folds distended with eggs lies between the acetabulum and the genital pore. On account of the large size of the reproductive organs, which almost fill the middle 2/3 of the body, the number of folds in this region is much restricted. The lateral longitudinal folds, which in most of the species of this genus run forward outside of the intestinal caeca, are very prominent in this species, extending forward for about 4 mm. from the posterior end or about 2/5 of the whole length of the worm, up to the anterior end of the posterior testis. My specimen in this particular shows a variation from Stafford's description and drawing. (Staf. 1902: 905 & taf. 33. fig. 2.) He writes:

"The posterior lateral folds of the uterus outside the ends of the intestinal caeca are short, extending only to the level of the posterior testis on one side and to its middle on the other."

My measurements for the eggs (Fig. VIII.) vary in length from 0.018 to 0.021 mm. and in width from 0.013 - 0.0156 mm. These are the smallest eggs recorded for the genus Haematoloechus, and are distinctly smaller than those of H. longiplexus, altho Stafford states that in his specimens they are of the same size, shape and color as in that species. The largest eggs measured for H. breviplexus are equal to the smallest of H. longiplexus but the average

is distinctly smaller for the former species.

The general size and shape of the body, the large size and peculiar shape of the testes, the ratio of the oral sucker to the acetabulum, the small size of the egg, the general arrangement of the uterus, the position, size, and number of the yolk glands and the characteristically shaped deeply lobed ovary have led me without hesitation to place my form in the species H. breviplexus. Stafford's material came from the lungs of Canadian frogs - Rana catesbiana and R. virescens - while my specimen was found in an Oklahoma toad - Bufo americanus. Other differences to be noted in my specimen were the absence of spines, the larger size of the oral sucker and acetabulum, the greater length of the lateral longitudinal folds of the uterus, and the smaller size of the eggs. In the first of these characteristics there seems to be variations in the other species also, the second and third differences seem to me to come within the normal limits of specific variation, and, in regard to the last point, as Stafford gives no measurements for the egg but simply states that they are the same size as those of H. longiplexus, it is probable that the small difference, noted above, was present in his specimens but escaped his notice.

6. Haematoloechus varioplexus. sp. inq. Stafford, 1902: 906.
1905: Pneumonoeces varioplexus. Stafford, 1905: 687.
1905: Pneumonoeces varioplexus. Klein, 1905: 64.

Specific diagnosis

Host Rana catesbiana Shaw. Canada.

Rather long worm, mounted specimen 10.5 mm. in length by 2 mm. in width.

In shape long elliptical or long oblong with narrowing ends, the anterior end being long and tapering.

Cuticula with spines.

Testes small rounded or short elliptical and some distance apart.

Small compactly rounded ovary.

Yolk glands extend into the posterior extremity of the body.

Lateral longitudinal folds of the uterus extend up to the posterior testis. Median portion of the uterus much folded.

The egg rather broad, an ellipse at both ends. In length 0.029 mm. x 0.018 mm.

This is the only one of Stafford's American species of which I have not obtained specimens. Of this species Stafford writes: (Staf. 1902: 906)

"I have obtained it in Toronto and Montreal, but I find that I have only few mounted specimens and imperfect notes; consequently I shall not describe it at length but trust to the drawing to illustrate its chief characteristics; I shall be glad to come upon it in numbers for greater assurance of its claim to specific distinction."

Considering the shortness of the description, the diagrammatic character of Stafford's drawing, and his admitted lack of material and observations, I feel like agreeing with him in suspending judgment on this species. I shall label it then as a

species inquirenda.

7. Haematoloechus similiplexus. Stafford, 1902: 907.

1905: Pneumonoeces similiplexus. Stafford, 1905: 687.

1905: Pneumonoeces similiplexus. Klein, 1905: 64.

Specific diagnosis.

Found in *Rana pipiens*, *Bufo lentiginosus*, and *R. virescens*.

Rather small spindle shaped worm from 4 - 7 mm. in length by about 1.6 mm. in width and 0.37 mm. in thickness.

Cuticle covered with scattered spines thinning out toward the posterior end, and absent back of the posterior testis, or thickly covered with spines.

Ventral sucker over three fourths the size of the oral sucker.

Testes small and rounded, the anterior being slightly the larger.

Pharynx 0.24 mm. long by 0.22 mm. broad.

Ovary rounded slightly elongated and smaller than testes.

Yolk glands from 5 - 10 groups on a side of from 18 - 25 follicles, extending to the very posterior end of the body.

Eggs large, averaging about 0.039 mm. by 0.019 mm.

Uterus with longitudinal folds outside of the intestinal caeca reaching to the anterior margin of the posterior testis.

Stafford reports this species (Staf., 1902: 907 - 908) from Canada in *Rana virescens* Kalm (Green frog) and from *Bufo lentiginosus* Shaw (American toad). In the first of these hosts

he found it equally plentiful with H. medioplexus. I found Haematoloechus similiplexus occurring rather rarely in Rana pipiens. In seventy five frogs of this species I found it in five hosts to the number of nine individuals, four being the largest number in one frog.

This form is rather sluggish and soon after being taken from the lung coils up. When placed in distilled water it throws out a part of its eggs, but soon shows a tendency to bend double at the region of the ventral sucker. The uterus is large in caliber and very much distended with large eggs, making the worm very opaque, and hard to study in toto and difficult to section.

It is spindle shaped, (Fig. III.) widest just back of the middle of the body tapering toward both ends, with the anterior end rather long and narrow. Stafford writes that these worms are more"inclined to be cylindrical than any of the preceding species but the older worms may be distinctly flattened." All of my worms were "distinctly flattened" measuring about 1/3 as much in thickness as in width. In size eight of my mounts ranged from 4.3 mm. - 6.6 mm. in length and from 0.9 mm. - 2.4 mm. in width, with an average size of about 5.4 mm. in length by 1.6 mm. in width. The cross section of one individual in the region of the anterior testis measured 0.9 mm. in width by 0.37 mm. in thickness.

The oral sucker (Fig. III.) measures about 0.37 mm. long by 0.34 mm. in diameter, and the ventral sucker is well developed measuring on the average about 0.32 mm. x 0.28 mm. x

0.18 mm. in depth. It is situated at from 1/4 to 1/3 of the distance from the anterior to the posterior end. The pharynx is rather large in proportion to the oral sucker measuring 0.22 mm. in length by 0.24 mm. in diameter. The oesophagus is about the length of the pharynx, and the intestinal caeca are as in the other species.

The cuticula and spines of the forms described by Stafford differ from those structures in my specimens. Stafford writes in this regard: (Staf., 1902: 907)

"The cuticle in preserved specimens is about 0.018 mm. thick and is regularly and thickly beset with spines, about 0.022 mm. in length leaning backward with a slight curvature and extending thru the whole thickness of the cuticle, with the points projecting beyond. Viewed from the surface, where one can see great numbers of them together, they appear to be in longitudinal and transverse rows, often 0.015 to 0.02 mm. apart, but sometimes even less, or more depending on the region and state of contraction of the animal. As is common they are most abundant at the anterior end."

The cuticula in my specimens is not as thick as in Stafford's account, and the spines are shorter and more scattered showing no regular arrangement into longitudinal and transverse rows. Measurements for the cuticula vary from 0.007 mm. to 0.012 mm., depending on the location and state of contraction. No spines were found longer than 0.013 mm. and in reasonably expanded portions I have never seen them nearer together than 0.025 mm. to 0.036 mm. Usually they are much more scattered than the above measurements would indicate. They thin out rapidly toward the

posterior end of the animal and the region behind the posterior testis is entirely smooth.

The testes (Fig.III.) are small rounded bodies, showing no signs of lobing, and situated, at quite a distance from the posterior end, just back of the middle of the animal on opposite sides of the body. The posterior testis is the larger, measuring in a specimen 4.5 mm. in length, 0.63 mm. in length by 0.44 mm. in width, while the anterior testis of the same specimen measured 0.5 mm. in length by 0.45 mm. in width. The testes fill almost the thickness of the body. In a cross section thru the posterior testis, which measured 1 mm. in width by 0.37 mm. in thickness, the testis showed a thickness of 0.33 mm.

The ovary (Fig. III.) is an elongated rounded body, averaging 0.46 mm. in length by 0.31 mm. in width, which lies to one side slightly back of the ventral sucker. As in the other species examined sexual amphitypy was present. In nine out of sixteen individuals the ovary was to the right. Stafford notes that the ovary was to the right in twelve out of twenty specimens. The small size and regularity of outline of the ovary and testes is very characteristic of H. similiplexus.

To the inside of the ovary lying diagonally, and in a ventral slightly posterior position to this organ is the receptaculum seminis, (Fig. III.) an elongated sac slightly longer than the ovary. It measures about 0.59 mm. by 0.31 mm.

The vitelline glands (Fig. III.) in this species number from 5 - 10 groups on each side of from 8 - 15 follicles in a group extending, from the region about half way between the oral

sucker and acetabulum to the very posterior end of the body. The size of the individual follicles varies from 0.065 mm. to 0.11 mm. There may be present both anterior and posterior median groups. Stafford notes that the vitellaria in H. similiplexus "extend completely into the posterior end of the body - in fact to the end of the intestinal caeca." His drawing, however, shows them extending only to the region of the posterior testis. Klein (Klein, 1905: 64) at the end of his description of a new species of this genus, sums up the specific differences of all the species of the genus Haematoloechus in a table for comparison. As one characteristic of H. similiplexus, in order to bring it into close relation with H. similis Looss, he gives the following:

"Dotterstöcke reichen nicht bis zum hintern Rand des hintern Hodens;" But he adds this note:

"Stafford gibt in seiner arbeit 1902 a an, dass die Dotterstöcke bei Pn. similiplexus bis ins Hinterende des Körpers, jedenfalls bis zum Ende der Darmschenkel reiden; diese Bemerkung stimmt jedoch nicht mit seiner eignen Abbildung überein, da letzere gleiches Verhalten zeigt wie bie Pn. similis."

That Klein made an error in judgment in picking this characteristic of H. similiplexus from Stafford's drawing rather than his description, is shown by the extent of the vitelline glands in my specimens of this species.

The shell gland which has the same structure as in the other species lies dorsal to the receptaculum seminis.

The folding of the uterus (Fig. III.) is characteristic. The coils of the uterus are thicker than in H. medioplexus, (Fig. IV.) are not so complicated and while in general are trans-

verse in direction in the posterior end show longitudinal foldings. They measure about 0.25 mm. in width and in all my specimens are very much distended with eggs. From its beginning in the shell gland the uterus passes for a short distance forward, and then goes to the right and loops down the right side in a slightly dorsal position to the region just back of the posterior testis, where it fills the space between the intestinal caeca. From the posterior end of the body it forms two longitudinal folds outside of the intestinal caeca, which reach almost to the anterior margin of the posterior testis. The uterus then ascends forward, ventral to the descending loops, passes between the testes to the right side of the body where it runs longitudinally for a short distance. Next it passes to the left side just behind the ovary, and taking its course around the ventral sucker fills with short transverse folds the region between the intestinal caeca up to the genital pore. The above description holds good for those individuals in which the ovary is to the right side of the body. The course is exactly reversed when the ovary is to the left.

The eggs (Fig. VII.) range from 0.038 mm. to 0.040 mm. in length by 0.017 mm. to 0.020 mm. in width. This is the largest size recorded for the eggs of the American species of Haematoloechus. This characteristic, with the large size of the acetabulum and the small size and regularity of contour of the ovary and testes make the identification of the species easy.

8. Haematoloechus medioplexus. Stafford, 1902: 908.
1905: Pneumonoeces medioplexus. Stafford, 1905: 687.
1903: Ostiolum formosum. Pratt, 1903: 34.
1905: Pneumonoeces medioplexus. Klein, 1905: 64.

Specific diagnosis

Occurs in Rana virescens, Bufo lentiginosus and R. pipiens.

Long graceful worms tapering to the anterior end and widest at the region between the testes.

An average mounted specimen about 8.6 mm. long by 1.4 mm. wide by 0.45 mm. thick.

Whole surface covered with a dense coating of short spines.

Oral sucker almost five times the size the acetabulum.

Pharynx large in proportion to the oral sucker.

The testes are large rounded bodies, smooth in outline or slightly lobed, of about the same size.

Ovary smaller than testes, elongated, rather irregular and usually lying transversely across the body.

Yolk glands 5 - 12 groups on a side of 5 - 12 follicles in a group, extending from a point about half way between the oral sucker and the acetabulum to just back of the posterior testis. Either the anterior or posterior group or both may lie in the middle of the body.

Uterus much folded, the general course of the folds being transverse, and the arrangement back of the testes being in a series of short transverse folds, usually with no longitudinal folds outside of the intestinal caeca.

Average size of eggs 0.026 mm. in length x 0.015 mm. in width.

My specimens of this species were easily identified as Haematoloechus medioplexus, agreeing with Stafford's description of

this form (Stafford, 1902: 908 - 910) in general size and shape, the small size of the acetabulum, the presence of a thick coating of small spines, the general size, shape and position of the reproductive organs, the characteristic folding of the uterus, and the size of the eggs. The thing which made this form most interesting to me was its close correspondence to Ostiolum formosum nov. sp. nov. gen. described by Pratt (Pratt 1903: 34 - 37.) Pratt's description differed from my specimens in the absence of spines, the position of the excretory system and the large size of the egg. The first difference can be easily explained by the tendency of the cuticula to slough off in these worms. In fact the slide of O. formosum obtained from Pratt showed that this had happened. A further study of this slide demonstrated that Pratt was in error in his description of the position of the excretory system and the size of the eggs. This confirmed me in my belief that H. medioplexus, of Stafford, my worms, and O. formosum all belong to the same species. A comparison of figures IV and V will show the identity of my specimens of H. medioplexus and O. formosum. This leaves, however, the question, Does this one species of lung flukes deserve to be placed in a new genus; that is, does it differ so much from the type species of the genus Haematoloechus, that it should be made the type of a new genus? This question I shall take up in some detail after I have described these forms.

In some of the hosts these worms were very abundant, even numbering ten to twenty in a lung. In the most heavily infected frog examined there were 18 in one lung and 22 in the other; the bulk of the contained trematodes being greater than that of the

lung tissue. This sets a record as far as I can find out for lung infection of frogs. Looss mentions an infection of 27 as being remarkable. The worms were not below the average size for the species. They were firmly attached by the oral sucker to the lining of the lung, with their bodies hanging free in the cavity, and in the heaviest cases of infection almost filling it. They are the most active of the worms of this genus which I have examined alive, showing a great power of expansion and contraction of the body and a very free movement of the long narrow anterior end. They are very tenacious of life, one lot living over night in normal salt solution. My best toto mounts were made from flukes left in distilled water until part of the eggs were given off. The worms treated in this way slowed up rapidly and in from ½ to 1½ hours evacuated a considerable proportion of their contained eggs, making it much easier to study the internal anatomy. In one of the specimens observed the penis was extruded soon after it was placed in distilled water and a quantity of sperms ejected. The length of the extruded penis was equal to about ½ the width of the body at this region.

The first thing to be determined in the study of the anatomy of this fluke is how to distinguish the dorsal and ventral surfaces. The acetabulum is so small and obscured by the folds of the uterus that in toto preparations it is almost impossible to find it. This caused me a great deal of difficulty in my early studies, and several errors in both Pratt's and Stafford's descriptions and drawings are caused, I believe, by a confusion of the surfaces. For this reason I have worked out from cross

and longitudinal sections the following criteria which may be used in the study of toto mounts to distinguish the dorsal from the ventral surface.

1. Acetabulum and genital pore lie on the ventral surface.

2. The intestinal caeca thruout their entire length lie somewhat dorsal-ward.

3. The vitelline glands lie near the dorsal surface.

4. The ovary and testes are slightly nearer the dorsal than the ventral body wall and coils of the uterus pass ventrally to their anterior and lateral margins.

5. The posterior part of the median excretory vesicle lies near the dorsal body wall.

H. medioplexus (Fig. IV.) is a long graceful worm, which tapers toward the anterior end and is widest in the region between the testes. The average length is about 8.5 mm. by 1.5 mm. in width by 0.45 mm. in thickness. There is much variation in size of adult worms due probably as Stafford notes to the fact that the worms attain sexual maturity before they have reached full size.

The cuticula measures 0.007 mm. in thickness and is densely covered with a thick coating of spines about 0.01 mm. in length and set firmly thru its entire thickness, pointing backward. Stafford very aptly describes them as appearing "under high objective and low ocular very much like a dense coating of short hair." Pratt makes the absense of spines in his specimens a very important diagnostic feature in O. formosum, in fact using it as one of the distinguishing characteristics of his new genus <u>Ostio-</u>

lum. In the toto preparation of O. formosum the cuticula had almost entirely sloughed off. Only in one place was I able to find a trace of it. This would account for the absence of spines. In figure IX I have shown under high magnification a portion of cuticula of one of my specimens of H. medioplexus showing the arrangement of the spines, and a portion from O. formosum showing a trace of the sloughing cuticula.

In H. medioplexus (Fig. IV.) the oral sucker measures about 0.39 mm. in length by 0.37 mm. in width. The ventral sucker is quite rudimentary, measuring only about 1/5 the size of the oral sucker and having very rudimentary musculature. In a mount 7.5 mm. in length it was situated 2.13 mm. from the anterior end and measured 0.095 mm. in width by 0.054 mm. with the lengths of the worm, by 0.06 mm. in depth. The pharynx is rather large in proportion to the oral sucker, measuring 0.27 mm. in length by 0.26 mm. in width. The short oesophagus and the intestinal caeca extending into the posterior extremity of the body correspond rather closely o those parts in the other species of this genus.

The excretory system (Fig. IV a, 1 -10.) in my specimens of H. medioplexus as is usual in this genus is Y shaped with a long median vesicle, and two short anterior crura. The median vesicle passes forward from the excretory pore along the dorsal median line up to the region of the posterior testis. It winds between the testes, taking its position farther in from the dorsal surface. In front of the anterior testis it resumes its original position close to the dorsal median line. At the posterior end of the ovary it divides into two crura, which wind forward and

ventralward along the sides of the ovary and shell gland and in front of these organs take their position parallel and ventral to the intestinal caeca. This position they keep until they terminate in the region of the anterior follicles of the yolk gland, about half way between the oral sucker and the acetabulum. Pratt's description for this system in O. formosum agrees with the one just given except that he describes the median vesicle as ventral and the crura as dorsal. This position would have been very unusual for a frog lung fluke and if true would have made a very strong point for generic distinction for the genus Ostiolum. That this description is based on an error in observation is shown very clearly from Pratt's own slide of O. formosum, in which the median vesicle of the excretory system can be made out running along the dorsal median line.

The ovary (Fig. IV, Fig. IV a, 7, 8, 11, 12, 13.) and testes have the usual position of those organs in the genus, the ovary being slightly to one side, the anterior testis on the other, and the posterior testis on the same side of the body as the ovary. Sexual amphitypy is present. In eleven out of eighteen of my mounts the ovary was on the left side. Stafford records in twelve out of nineteen specimens that the ovary was to the right.

The testes are large, rounded bodies lying on opposite sides of the animal just back of the middle, the anterior testis always being on the opposite side of the body from the ovary. They are rounded or slightly lobed organs varying in size and shape, and of about the same size. They vary in length from 0.6 - 1.11 mm., and in width from 0.6 - 0.9 mm. They average about

0.36 mm. in thickness. They lie slightly nearer the dorsal side of the animal and almost fill the space dorso-ventrally. In the type specimen of O. formosum the testes were slightly lobed and measured 1.11 mm. in length by 1.04 mm. in width. In three out of eighteen of my mounts the testes showed lobing and in one the lobing was as deep as in Pratt's specimen.

The vasa efferentia (Fig. IV a, 8,9,10,11.) run forward from the anterior ends of the testes at either side of the mid dorsal line up to the region of the ovary and shell gland, where they pass ventrally to join into the vas deferens just anterior to the ovary. The width of these tubules is 0.012 mm. The vas deferens at the junction of these ducts widens to 0.07 mm. and functions as a seminal vesicle for the greater part of its length. It is enclosed for the whole distance in a cirrus sac, the posterior part of which lies in contact with the walls of the enclosed tube. The anterior part for about 0.2 mm. functions as a short cirrus which in one instance already mentioned was protruded. Just back of the cirrus is the short ductus ejaculatorius, around which cluster the prostate glands. (Fig. IV c.)

The ovary (Fig. IV.) is an elongated irregular organ lying just back of the acetabulum to one side or the other of the median line, usually lying obliquely with the long axis of the body. It varies much in size. The smallest ovary measured was 0.36 mm. with the length of the animal by 0.45 mm. with its width, and the largest was 0.63 mm. with the length by 0.77 mm. with the width. The thickness is about 0.3 mm.

The oviduct (Fig. IV b.) is a short narrow duct about 0.009 mm. in width, which leaves the ovary dorsally from its

posterior lateral margin and runs thru the shell gland, and becomes the oötype. Within the shell gland it receives the ducts from the seminal receptacle, and the yolk glands. (Fig. IV b.)

The seminal receptacle (Fig. IV, IV a, 9, IV b.) in these worms is very large and filled with sperms. It lies directly back of the ovary rather more to the center of the body and is of about the same general size and shape as the ovary. It varied in ten specimens from 0.53 mm. - 0.67 mm. in length and from 0.37 mm. - 0.61 mm. in width. The transverse axis is usually the longest. The duct of the seminal receptacle (Fig. IV b.) is short about the width of the oviduct, and leaves the anterior dorsal end of this organ, and meets the oviduct within the shell gland.

The description of Pratt for the shell gland is so good and applies so directly to my forms that I shall copy it exactly.

"The shell gland is an extensive group of glandular cells situated between the anterior end of the ovary, and the receptaculum seminis, and enveloping the proximal portion of the uterus, and also portions of the oviduct and median yolk duct. Laurer's canal could not be found." Stafford makes no mention of the shell gland in H. medioplexus. After reading Goldschmidt's paper pn the "Yolk Glands, Shell Glands and Shell Formation in Trematodes," (Goldschmidt, 1909) in which he ascribes the shell formation not to the so-called "Shell-glands," but to the vitelline glands, I was struck by the absolute inadequacy of such a gland as the so-called "shell gland" of the lung fluke to furnish the shells for the multitude of eggs which distend the uterus. In the ducts of the vitellaria I could distinguish the same shell

droplets that were described by Goldschmidt. This has lead me to believe that in the frog lung flukes as well as in the trematodes described by Goldschmidt, the so-called vitelline glands secrete the material which forms the egg shells.

The groups of follicles of the vitelline glands (Fig. IV.) are located near the dorsal surface of the animal outside the intestinal caeca ranging from the region about half way between the oral sucker and the acetabulum, to back of the posterior testis. The number of groups varies from five to twelve on a side and from five to twelve follicles in a group. The individual follicles vary from 0.09 mm. to 0.19 mm. in length. The anterior or posterior group or in some cases both may be located in the mid dorsal region between the intestinal caeca. There is, however, quite a variation in the number and position of the vitelline glands. In _H. medioplexus,_ however, they are characterized by the small number of follicles in a group and the large size of the follicles. The ducts of these glands collect from the individual follicles and run down each side from the anterior and posterior ends and meet in the region posterior to the shell gland to form two short transverse yolk ducts (Fig. IV, b.) These pass into a median yolk duct, which is enlarged to form the yolk reservoir. This reservoir empties into the oviduct just after it has received the duct from the seminal receptacle. (Fig. IV, b)

The oviduct after receiving the ducts from the seminal receptacle, and vitelline glands becomes the oötype, (Fig. IV, b.) a short duct 0.015 mm. wide which takes its course ventrally thru the shell gland. This part of the duct is distinguished by a characteristic musculature. From the shell gland the oötype passes

into the uterus, (Fig. IV, IV a, 1-10.) which runs ventrally for a short distance, then winds backward between the testes to the region behind these organs where it courses in short transverse folds down one side to the posterior end of the body and back up the other to the region of the posterior testis, filling the space between the intestinal caeca but entirely enclosed by them. The uterus then passes anteriad between the testes ventrally in the region of the seminal receptacle, and the ovary and from there up to the genital pore in a series of complicated transverse folds. The folds of the uterus are voluminous, somewhat irregular, but in general course transverse, being throughout their entire length between the intestinal caeca. In adult specimens for its entire length the uterus is distended with eggs. As the average width of the uterus is about 0.15 mm., and its total length must be many times the length of the body. The enormous number of eggs present in one specimen may be surmised. The fact that the uterus is entirely within the intestinal caeca is one of the most important distinguishing features of H. medioplexus, and in fact is one of the chief characters on which Pratt bases his new genus Ostiolum. Seely notes this condition also in the forms of this genus which he describes under the name Pneumonoeces complexus. (Seely, 1905.) An interesting variation and of considerable importance, since it shows that the above characteristic is not absolutely constant for this species, appears in two of my specimens. In thse forms, there are present short longitudinal folds of the uterus at the posterior end, outside of the intestinal caeca. (Fig. VII.) These were the oldest specimens studied of this species, judging

by the great complexity of the folding of the uterus and the great mass of eggs present.

The eggs (Fig. VIII.) are oval in outline, disc shaped, and have one end, at which the operculum is found, slightly more pointed than the other. The color is dark brown, giving a dark mottled color. In measurements of fifty eggs from different worms I found the size to vary from 0.022 mm. x 0.014 mm. up to 0.029 mm. x 0.018 mm. The average size was found to be 0.026 mm. x 0.015 mm. Stafford gives the size of the eggs for H. medioplexus as 0.028 mm. x 0.018 mm., while Pratt gives the size in O. formosum as 0.039 mm. x 0.017 mm. In the specimen of O. formosum which I obtained from Pratt, I found the size of the eggs to be the same as in my forms, no eggs measuring more than 0.027 mm. in length. Pratt has since re-measured the eggs for O. formosum and has obtained about the same results as I have above recorded in H. medioplexus. He ascribes his original mistake to a confusion of notes.

This study has convinced me that the long lung flukes which I have collected from the lungs of Rana pipiens are correctly diagnosed as H. medioplexus, and that Pratt's form described under the name Ostiolum formosum belongs to the same species. Having established then the identity of these three forms, the next question to be taken up is, Does this species show sufficient differences from the species included under the genus Haematoloechus to warrant its being made the type species of a new genus? If not, Ostiolum formosum should be included within the genus Haematoloechus, and as Stafford's specific name antedates Pratt's, Ostiolum formosum would be considered as a synonym of Haematoloechus medioplexus.

Pratt establishes this new genus Ostiolum with the type species O. formosum from the study of several specimens of this long lung fluke on account of the following differences. I will quote his exact words.

"It (Ostiolum formosum) differs principally from Haematoloechus in the position of the acetabulum which is farther forward than in that genus, the size of the testes which are much smaller than in Haematoloechus, in the arrangement of the uterine folds, which have a general longitudinal direction in Haematoloechus, and in the length of the excretory vesicle which extends much farther forward than in Haematoloechus. In that genus also the worms are often covered with spines, while in Ostiolum these structures did not appear in any of the specimens examined by me."

How the testes can be much smaller than in Haematoloechus is hard to understand as in Pratt's type specimen of O. formosum which is 1.5 mm. wide the anterior testis is 1.15 mm. wide, and has practically the thickness of the body of the animal. Besides a review of the specific diagnoses included in this paper will show that there is much variation in this respect in all the species included under the genus Haematoloechus. The folding of the uterus shows a distinct difference, but as the uterus fills practically all the spaces among the organs, its course is rather variable, depending on slight differences in arrangement and size of the organs, and hardly the thing on which to base a generic distinction. My specimens, in which short longitudinal folds of the uterus are present seem to me to further invalidate this point as a difference of generic rank, since it shows that even within the

species there may be individual variations in this regard. Slight differences in the position of the acetabulum, and the length of the excretory vesicle seem to me to be of specific rather than generic value. In fact I can see more reasons why H. longiplexus or H. breviplexus should constitute the type species of a new genus, than H. medioplexus. Therefore I believe that Pratt's claim of generic rank for Ostiolum is far from established. I will agree then with Stafford that this type of frog lung flukes belongs within the genus Haematoloechus and since Stafford's specific name antedates Pratt's the trematode under consideration in my opinion should be known as Haematoloechus medioplexus.

9. Haematoloechus capyristes. New name for the trematode Pneumonoeces capyristes. Klein, 1905: 60.

Specific diagnosis

Host Rana hexadactyla Less. from India.

In shape depending on the state of contraction from bluntly rounded and of equal width at both ends to spade formed - "spatel-formig"-

Cuticula without spines.

Oral sucker twice as large as the acetabulum - 0.324 mm. - 0.156 mm.

Testes irregularly shaped, rather elongated, the posterior being the larger, close together and overlapping.

Cirrus sac short and pear-shaped.

Ovary much smaller than the testes elongated oval in shape, in length 0.516 mm. x 0.32 mm. in width.

Yolk glands 6 - 8 groups with 4 - 8 follicles in a group arranged along each side of the body. The anterior group in the mid dorsal line half way between the mouth and the ventral sucker, posterior group median near posterior end of the body.

Lateral longitudinal folds of the uterus extend to the region between the ovary and anterior testis.

Eggs are dark brown, oval, in length varying between 0.028 mm. and 0.0308 mm,,and in width between 0.014 mm. and 0.018 mm.

Two points are worthy of note in regard to this species, the great variation in shape of the different specimens, and the short pear shaped cirrus sac. The specimen figured in the first drawing of this species (Klein, 1905. taf. 5, fig. 1.) was compressed under a cover while alive. This would account for the very much widened anterior end. All the worms of this genus are very flexible and the shape of preserved material depends much upon the method of handling.

The shape of the cirrus sac of this species would alone serve to distinguish it from any of the other species of this genus, this being the only case in which the cirrus sac is not rather long and narrow.

10. Haematoloechus complexus sp. inq. New name for trematode Pneumonoeces complexus. Seely, 1906: 248.

Specific diagnosis

From the mouth of Rana pipiens. North Carolina.

Elongated worm 5 - 8 mm. in length, 1.7 mm. in width

and 0.71 mm. in thickness. Widest just in front of the middle, tapering slightly to a blunt posterior end, and rapidly toward the anterior end.

Oral sucker very slightly larger than the acetabulum. Oral sucker is 0.4 mm. in diameter, and the ventral sucker 0.38 mm.

Cuticula without spines. There was, however, no examination of fresh material in this form.

Testes irregularly ovate in outline and somewhat lobed, and slightly overlapping.

No cirrus or cirrus sac.

Genital pore just back of the pharynx and to the side of the median line.

Ovary somewhat elongated and oval in outline. 0.7 mm. in length and 0.32 mm. in width.

The receptaculum seminis about ½ as large as the ovary.

Yolk glands of 5 or 6 clusters of six to twenty follicles on a side, ventral in position and extending from the pharynx to to the hind end of the posterior testis.

No lateral longitudinal folds of the uterus.

Egg dark brown in color and 0.029 x 0.014 mm.

In his conclusion (Seely, 1906: 252) Seely relates this worm most closely to H. similiplexus, but points out differences in the size of the eggs, in the arrangement of the folds of the uterus and in the lack of spines. He explains the presence of this worm in the mouth of the frogs by the fact that the hosts were examined after the frogs were killed in chloroform and left

for some time in the killing jar, so that probably they are lung flukes, which have migrated into the mouth. This brings up the absurdity of killing frogs which are to be examined for parasites in chloroform, when it is so much easier on the frog, the manipulator and the parasite, to use a needle at the back of the neck.

Strange to say Seely makes no special mention of three rather important characteristics of H. complexus as described. According to his description and figure (Seely, 1906: 248 - 252) this worm has no cirrus or cirrus sac, a genital pore to one side of the median line, and the yolk glands on the ventral side of the body. In these particulars Seely's lung fluke stands as unique among all the species previously ascribed to the genus Haematoloechus. The position of the genital pore might be ascribed to distortion. Seely's description was based on "several preserved specimens which were received from North Carolina marked, 'From the mouth of Rana pipiens.'" Seely seems to ascribe no particular value to the differences noted above. I would be inclined to ascribe them to mistakes in observation due perhaps to the poorness of the material. Until further observations can be made on this species I feel that judgment must be suspended, and it labeled a species inquirenda.

The species of the genus Haematoloechus, discussed above, have been reported from Germany, India, Canada and the United States as far south as North Carolina and Oklahoma from various species of frogs and toads. A given host even in the same region may harbor several different species, and a given species of trematode may infest several different hosts. For

example, Stafford reports H. longiplexus, H. breviplexus and H. varioplexus from Rana catesbiana, and I have found in Rana pipiens specimens of H. longiplexus, H. similiplexus, and H. medioplexus. Stafford reports H. medioplexus from Rana virescens and Bufo lentiginosus while I have found this species in abundance in Rana pipiens. H. breviplexus, which Stafford reports from Rana catesbiana in Canada has been found in an Oklahoma toad, - Bufo americanus.- An interesting condition was found in the case of the infection of Rana pipiens with H. medioplexus, H. similiplexus and H. longiplexus. Two of these forms would sometimes be found in the same frog and even in the same lung. The data on this is given in the following table.

Hosts	H. medioplexus	H. similiplexus	H. longiplexus
No. 16	1	3	
" 18	1	2	
" 20	2		1
" 37		1	1
" 38	1		1
" 47	1	1	
" 57	1		2

In hosts numbers 47 and 57 both kinds of flukes were found in the same lung. The above table shows that in none of the frogs heavily infected with H. medioplexus were specimens of either of the other species present. My data on the amount of infection of these three species, i. e. H. medioplexus, H. similiplexus and H. longiplexus, show that the first of these three species is by far the most common lung fluke in R. pipiens, and that the other two are compara-

tively rare. This has suggested the hypothesis that H. longiplexus and H. similiplexus may be the dominent lung flukes in other species of frogs and that the infection of R. pipiens with them might be considered as merely occasional. Stafford writes of H. longiplexus as by far the commonest lung fluke of R. catesbiana. Only a long series of observations can clear up this point.

Any detailed comparison of the different species of this genus or any attempt to work out their relationships is in the present state of our knowledge very unsatisfactory. The descriptions of some of the species are very short, rather inaccurate and too general, and often some of the most important diagnostic characters are not well worked out. The points which have been of most value to me in diagnosis have been the character of the folding of the uterus, the size, shape and position of the reproductive glands, the comparative size of the oral sucker and the acetabulum, the size and shape of the body, and the size of the eggs. The species I have studied particularly are of very characteristic appearance. In fact H. longiplexus and H. similiplexus and H. medioplexus can easily be sorted out either while living or in preserved condition. The study of the cuticula has proven very unsatisfactory, and even when intact it seems to vary in the presence and distribution of spines in the same species from different localities. For example, Stafford reports spines for H. breviplexus, yet in my specimen from an Oklahoma toad even in places where the cuticula seemed to be intact I could find no trace of spines. Stafford also reports that even in freshly killed specimens of H. longiplexus no spines were present, while I have always found them present in my specimens of this species. H. similiplexus also offers an interesting variation

in this regard, for Stafford reports a dense coating of spines all over the body, while I have found scattered spines only in the anterior 2/3 of this animal. Further investigations with abundance of material are needed to clear up this point.

In many points which require careful observation the specific differences are entirely undescribed for this group. For example I find a distinct difference in H. longiplexus and H. medioplexus in the relations of the end ducts of the reproductive systems and excretory systems. Stafford states that the excretory system in his five species of Haematoloechus is of little value for specific diagnosis. Differences in the vitellaria are probably characteristic of all the species of this genus but have only been worked out for part of them. It seems to me rather rash to infer that any system in a genus which has not been worked out in some detail offers no important variations.

An account of the fragmentary character of our knowledge of most of the species it is impossible to express the relationships within this genus with any degree of accuracy. The following key will perhaps be of aid in the identification of the forms.

Key to the genus Haematoloechus.

I. Longitudinal folds of the uterus outside the intestinal caeca very long, extending forward to the region of the pharynx.

H. longiplexus,

II. Longitudinal folds of the uterus present outside the intestinal caeca but never extending farther forward than the ovary.

A. Testes somewhat irregular in outline, close together, and overlapping for part of their length.

1. Testes and ovary very deeply lobed. Eggs very small, about 0.019 mm. x 0.014 mm.

H. breviplexus

2. Testes and ovary irregular but not lobed, cirrus sac pear shaped. Egg 0.029 x 0.016.

H. capyristes

B. Testes round or oval in outline, distinctly separated, and not overlapping.

1. Cuticula covered with wart like protuberances. Eggs very large, 0.055 mm. - 0.029 mm. H. asper. sp. inq.

2. Cuticula smooth. Eggs 0.029 mm. x 0.016 mm.

H. variegatus.

3. Cuticula with spines.

a. Yolk glands short not extending behind the posterior testis. Median portion of the uterus not greatly folded. From 7 - 10 mm. in length. Eggs, 0.038 x 0.019 mm.

H. similigenus

b. Yolk glands extending into the posterior extremity of the body. Median portion of the uterus much folded. Large in size, about 10 mm. in length. Eggs 0.029 mm. x 0.018 mm.

H. varioplexus. sp. inq.

c. Yolk gland extending into the posterior extremity of the body. Median portion of the uterus rather complexly folded. Small

in size, 3 - 8 mm. long. Egg,
0.039 mm. x 0.019 mm.
H. similiplexus.

III. No longitudinal folds of the uterus outside the intestinal caeca.

A. Acetabulum about 1/5 the size of the oral sucker.
H. medioplexus.

B. Acetabulum over $\frac{3}{4}$ the size of the oral sucker.
H. complexus sp. inq.

Finally, Looss' generic diagnosis with the increase in the number of species which to my mind must be placed in the genus Haematoloechus, has become inadequate to express the generic relationships. (Looss, 1899: 600 & 601.)

The following diagnosis expresses in my opinion more accurately the limits of the genus Haematoloechus at the present state of our knowledge.

Diagnosis of the Genus Haematoloechus.

Medium sized worms, elongated with an elliptical cross section, and the anterior end somewhat slenderer than the posterior.

Acetabulum weakly developed and always smaller than the oral sucker.

Cuticula delicate, easily sloughed off, without spines or with many small irregularly arranged spines.

Digestive system, having pharynx, a very short oesophagus and the intestinal caeca, extending into the posterior extremity of the body.

Main trunks of the excretory system Y shaped with a

long median vesicle and short crura.

Genital pore in the mid ventral line just back of the oral sucker.

Cirrus sac very elongated extending back to acetabulum, having side folds and enclosing a large seminal vesicle for most of its length, or much shorter and pear shaped.

In the anterior part of the cirrus sac, a short ductus ejaculatorius, a scarcely distinguishable pars prostica, surrounded by prostate glands and a short protrusible cirrus.

Testes variable in shape, the one on the same side as the ovary being the more posterior and usually a little the larger.

Seminal receptacle large and just back of the ovary.

Laurer's canal absent.

Ovary just back of the acetabulum, smaller than the testes, variable in shape, and lying slightly to one side of the body of the animal.

Yolk glands follicular, along each side of the body, outside or just inside the intestinal caeca, and often either the anterior or posterior gfoups or both may be median.

Uterus very long and voluminous, and thruout its whole course packed with eggs. Ascending and descending branches pass in between the testes, Usually lateral longitudinal folds lie outside the intestinal caeca and a very complicated series of transverse folds fills the anterior end.

Eggs, brown,. oval, disc-shaped, varying between 0.019 - 0.05 mm. in length by 0.013 mm. - 0.03 in width.

In lungs of Amphibians. Type species <u>H. variegatus</u> <u>Looss</u>.

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Fig. IX. A comparison of the cuticula in H. medioplexus and O. formosum. High magnification.

Abbreviations Used in Figures.

O. s. - Oral sucker.

Ph. - Pharynx.

Oes. - Oesophagus.

Int.caec. - Intestinal caeca.

V. g. - Vitelline glands.

U. - Uterus.

Ov. - Ovary.

R. s. - Receptaculum seminis.

T 1. - Anterior testis.

T 2. - Posterior testis.

A. - Acetabulum.

Ex. v. - Excretory vesicle.

Cir. s.- Cirrus sac.

Pen. - Penis.

Pr. g. - Prostate glands.

D. e. - Ejaculatory duct.

S. v. - Seminal vesicle.

Ex. c. - Excretory crura.

V. d. - Vas deferens.

Oöt. - Oötype.

Sh. g. - Shell gland.

V. e. - Vasa efferentia.

Ac. - Acetabulum.

Vg. r. - Vitelline reservoir.

Ov. d. - Oviduct.

R. s. d. - Duct of the Receptaculum seminis.

G. p. - Genital pore.

Vag. - Vagina.

Cir. S. - Cirrus sac.

E. d. - Ejaculatory duct.

P. p.-Pars prostica.

Fig. I

H. longiplexus. Dorsal View. Toto Mount.

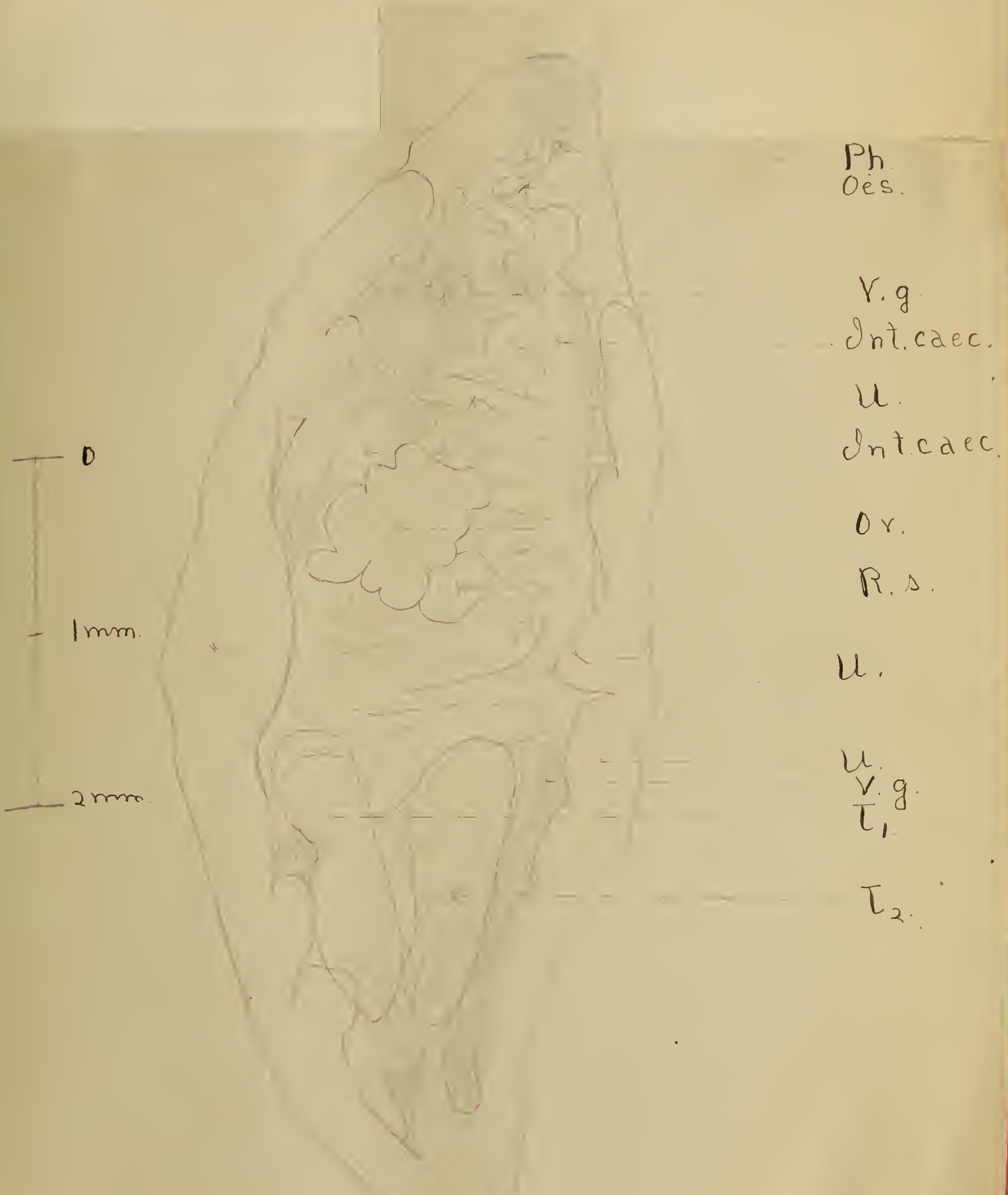


Fig. I, a.

A Series of Cross Sections of H. longiplexus.

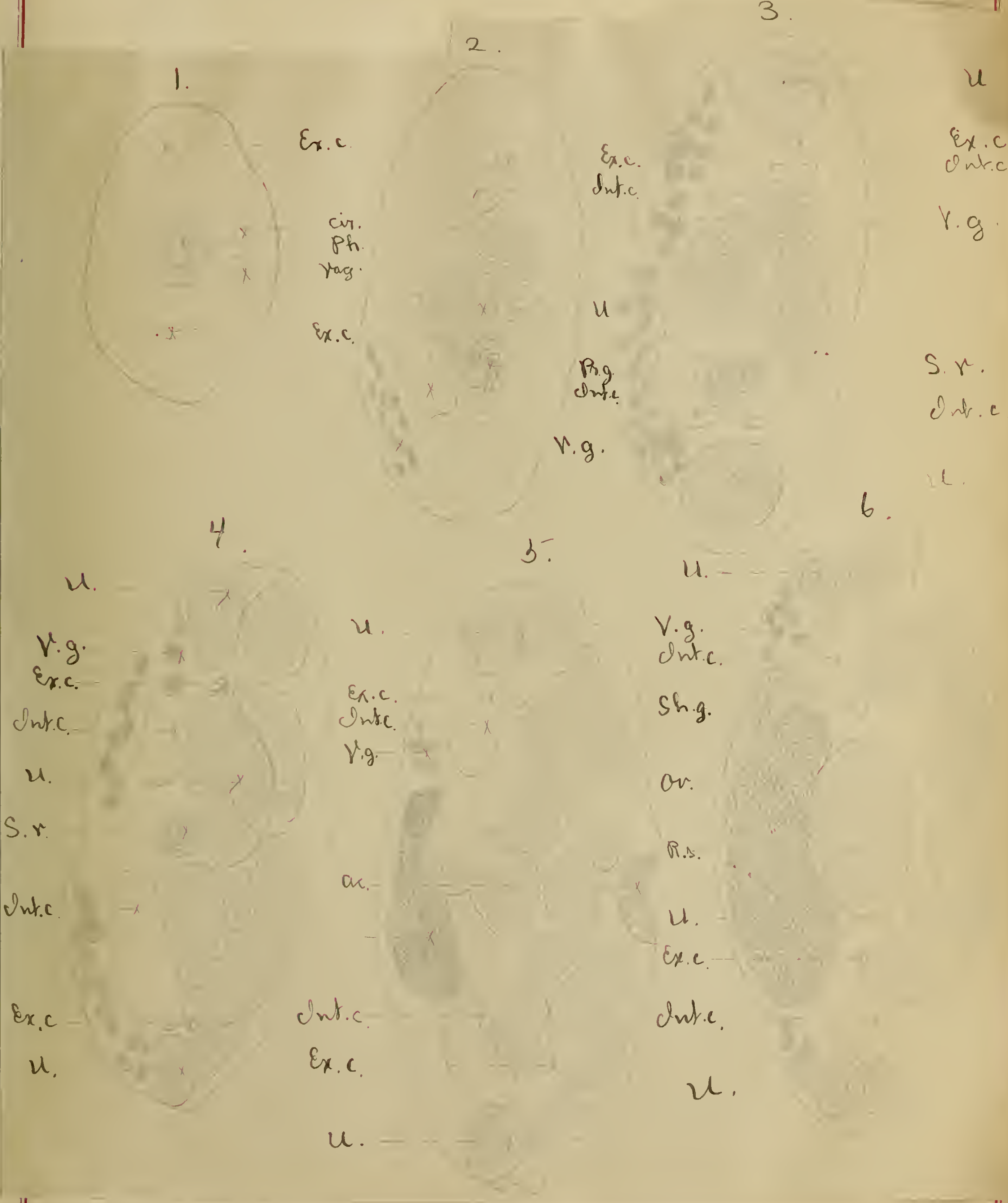
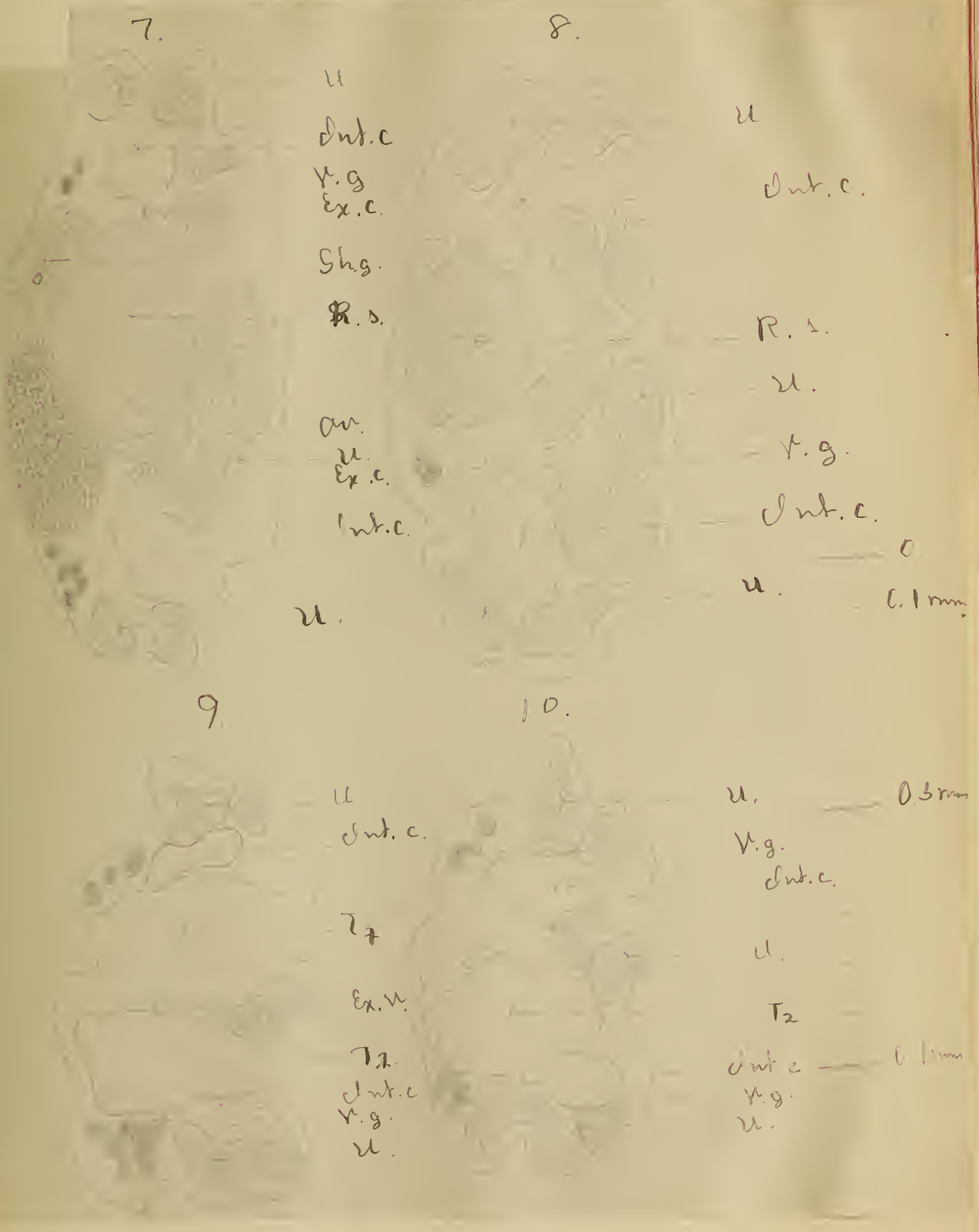


Fig. I, a. Cont.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R.s.
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Int.c.
T1
Ex.v.
T2.
Int.c
V.g.
u.
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u.
0.3mm
V.g.
Int.c.
u.
T2
Int.c
0.1mm
V.g.
u.

Fig. I, b.

Connections of the Female Reproductive System
H. longiplexus. Dorsal View. Diagrammatic.

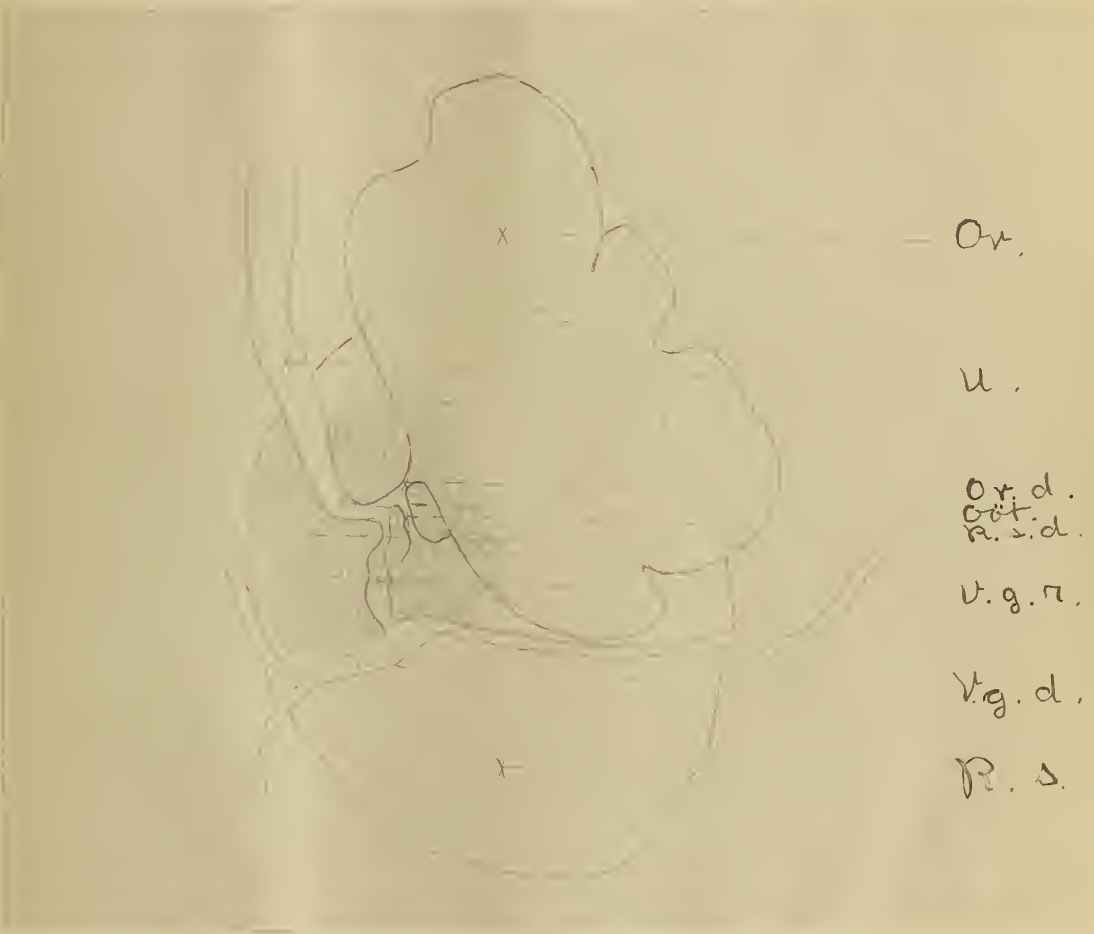


Fig. I, c.

End Passages of the Reproductive Systems of H. longiplexus.

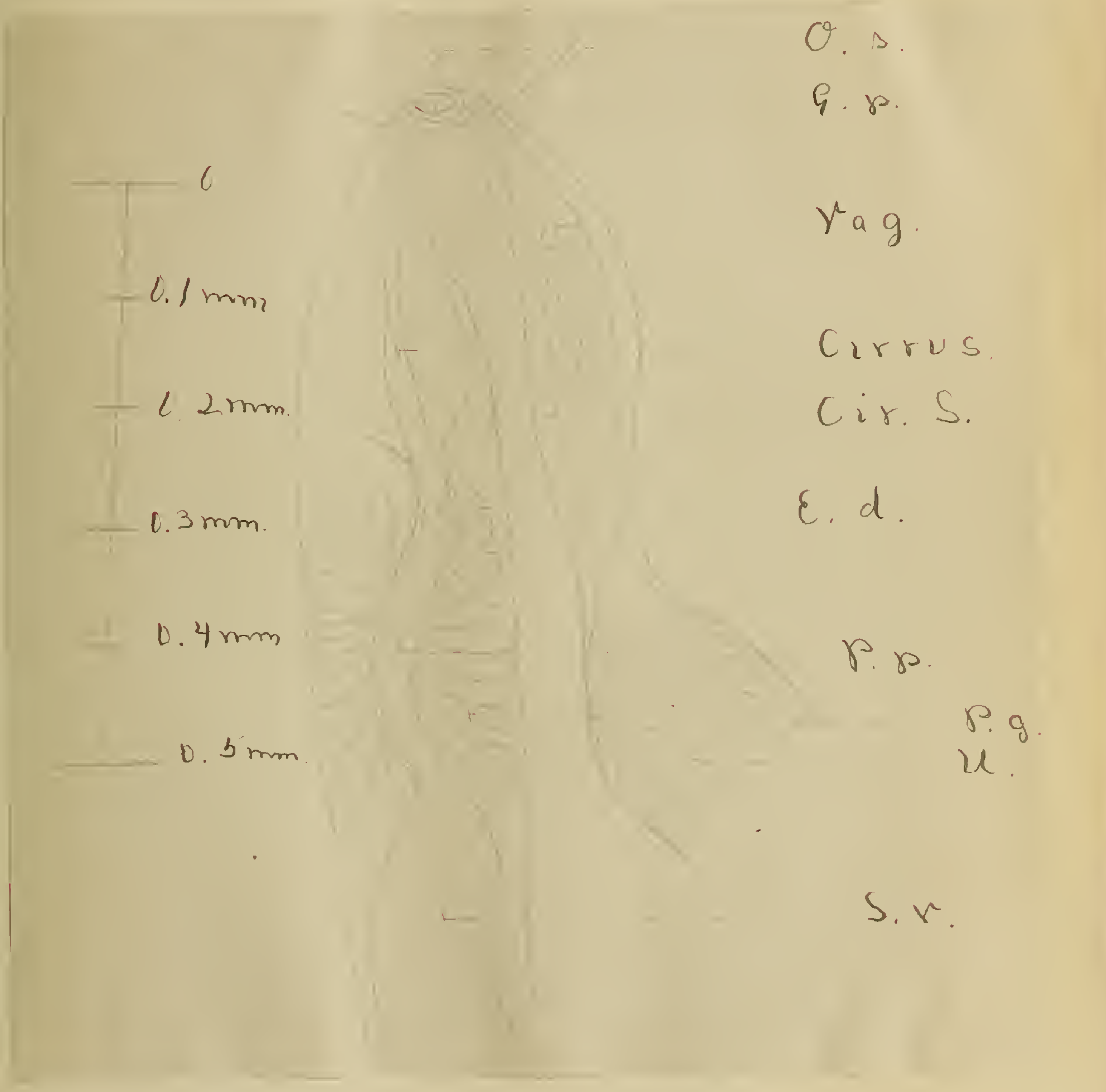


Fig. II.

H. breviplexus. Dorsal View. Toto Mount.

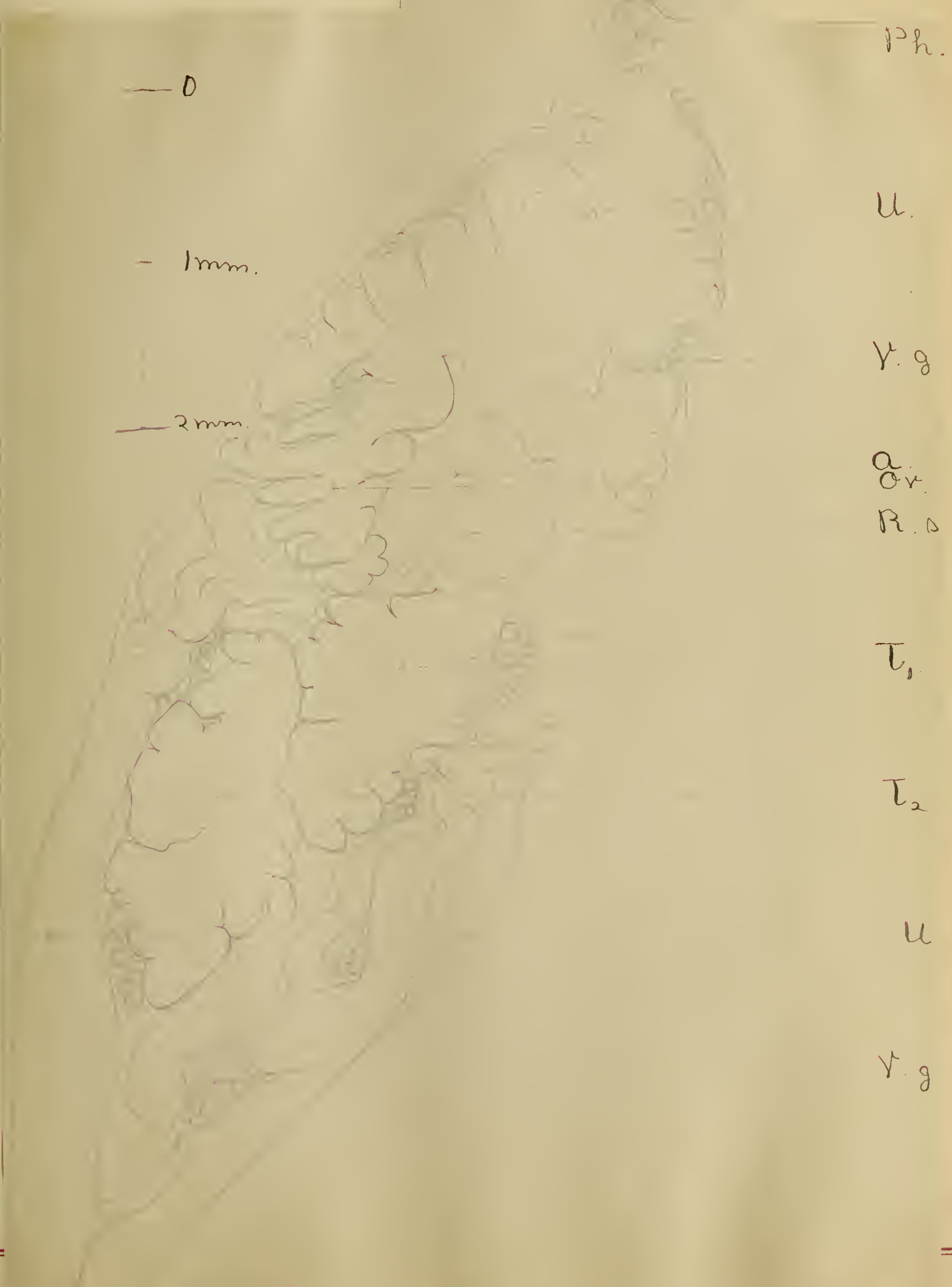


Fig. III.

H. similiplexus. Dorsal View. Toto Mount.

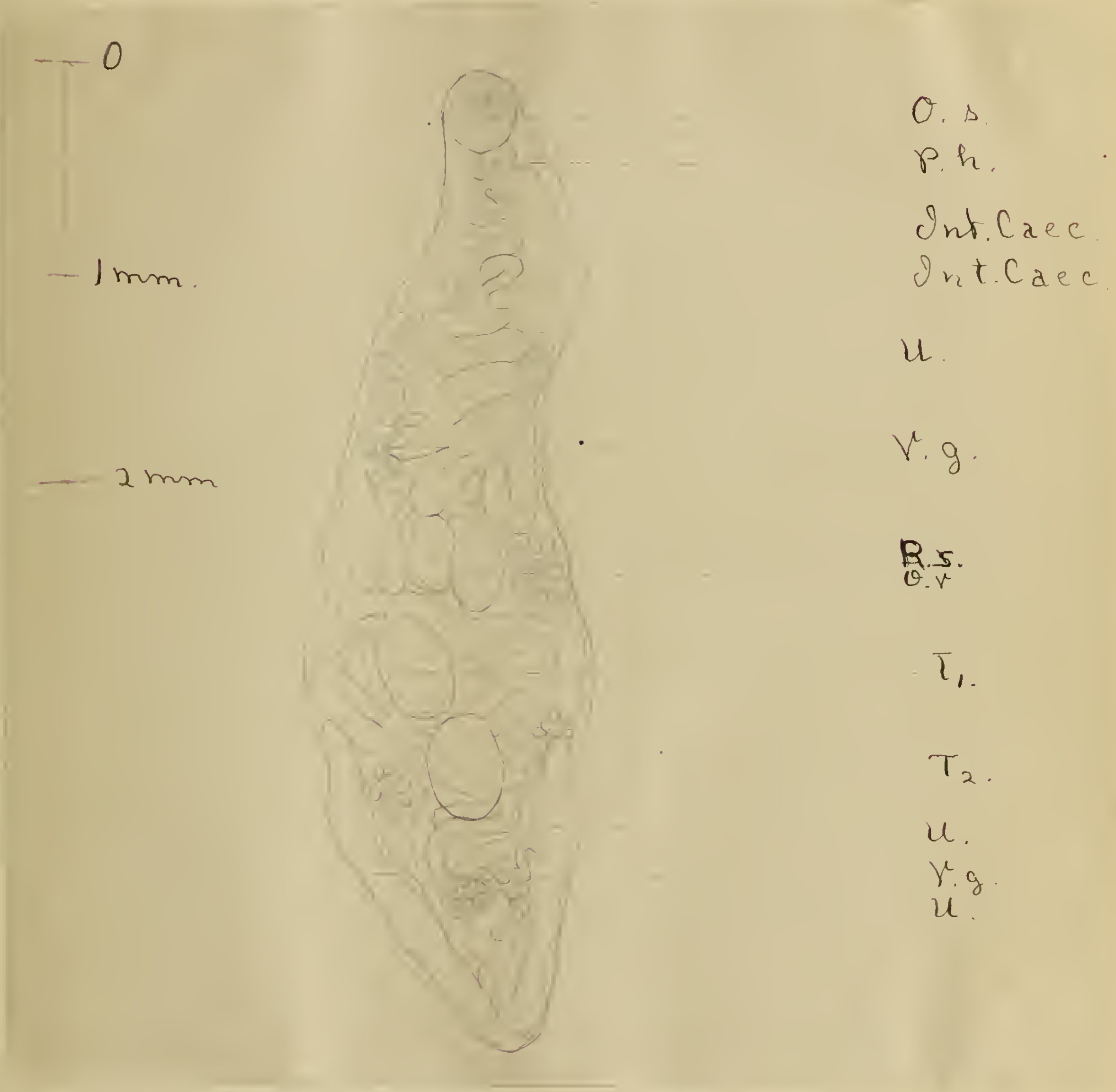


Fig. IV.

H. medioplexus. Dorsal View. Toto Mount.

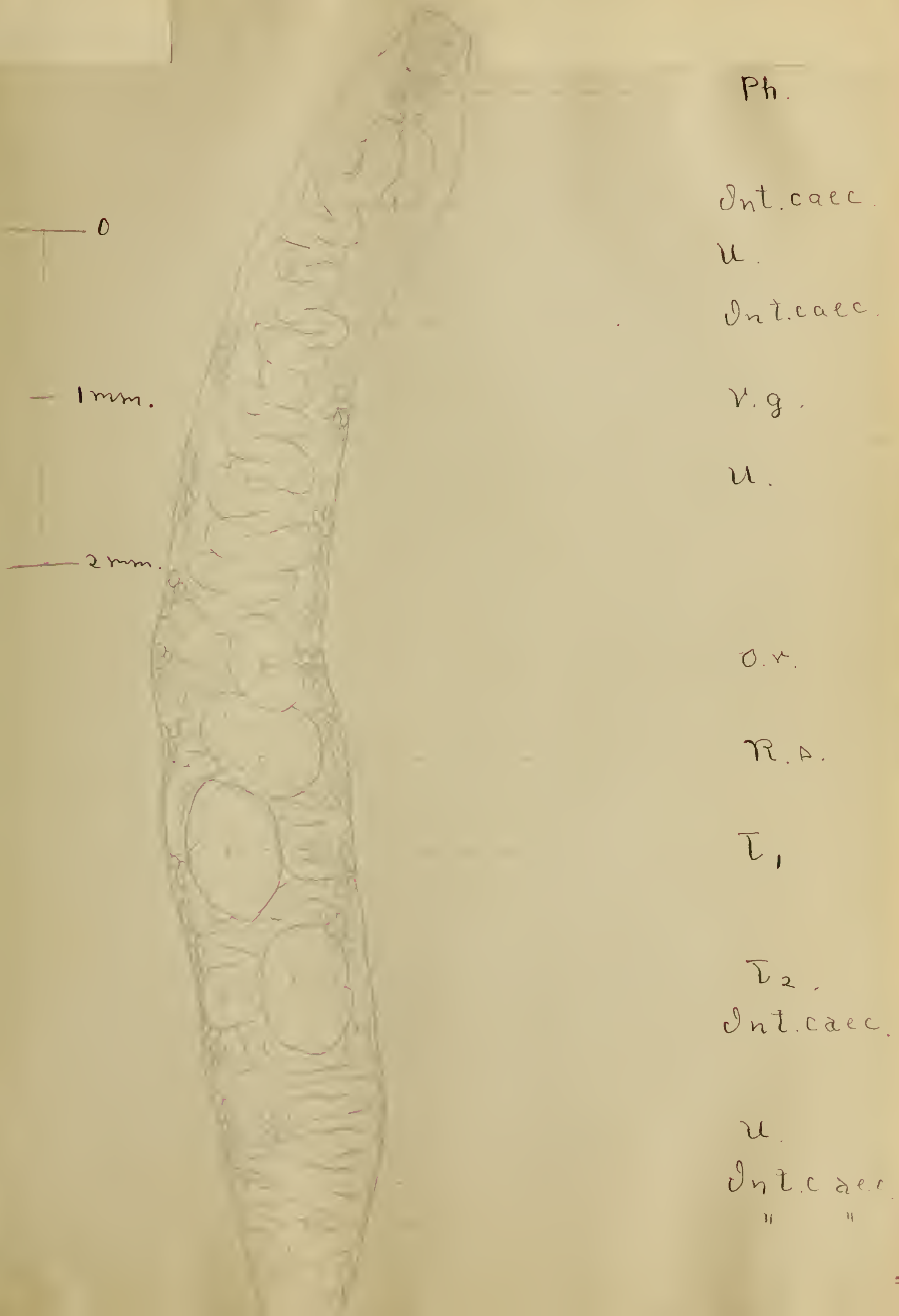


Fig. IV, a.

A Series of Cross Sections thru Different Regions of the Body in H. medioplexus.

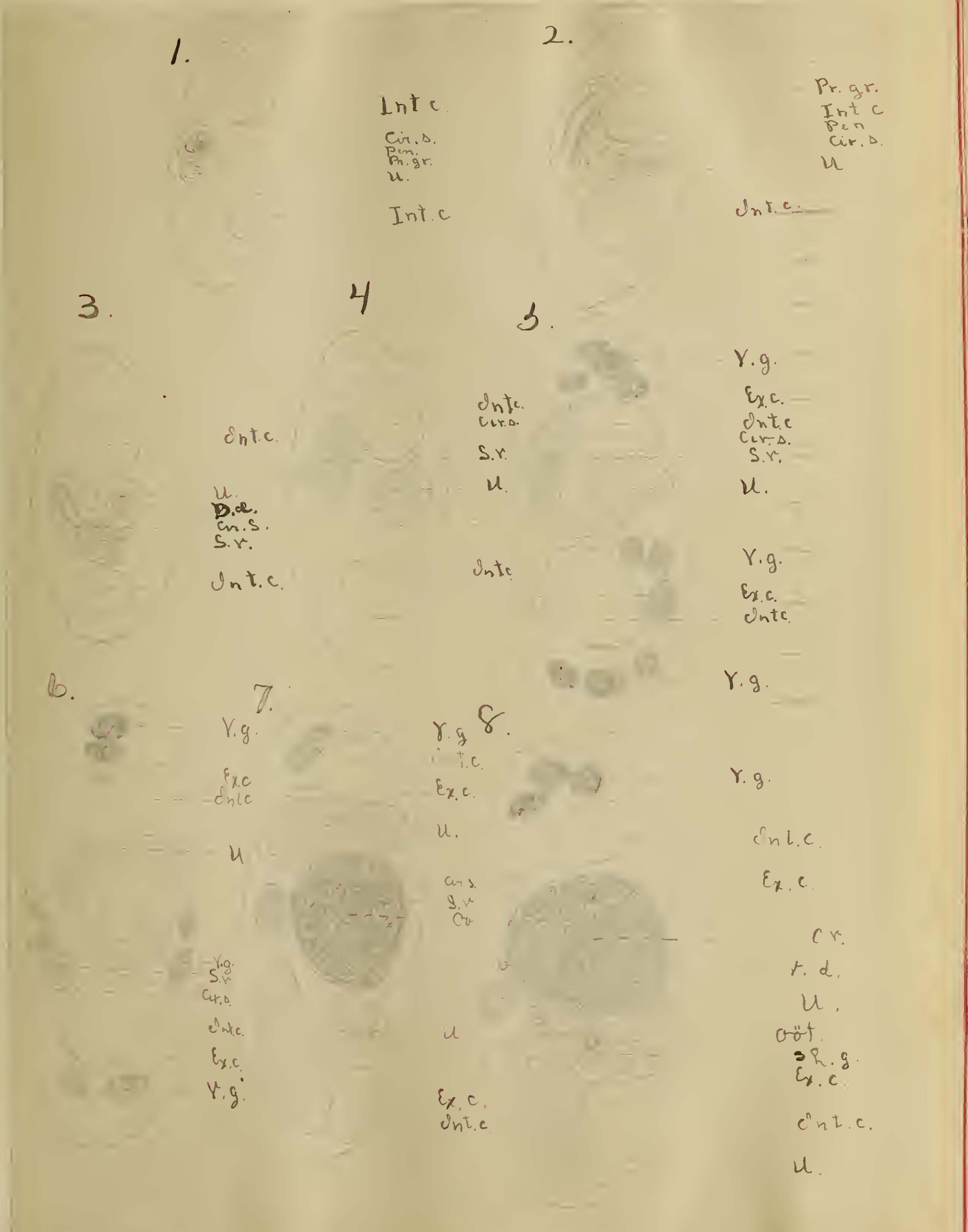


Fig. IV, a. Cont.

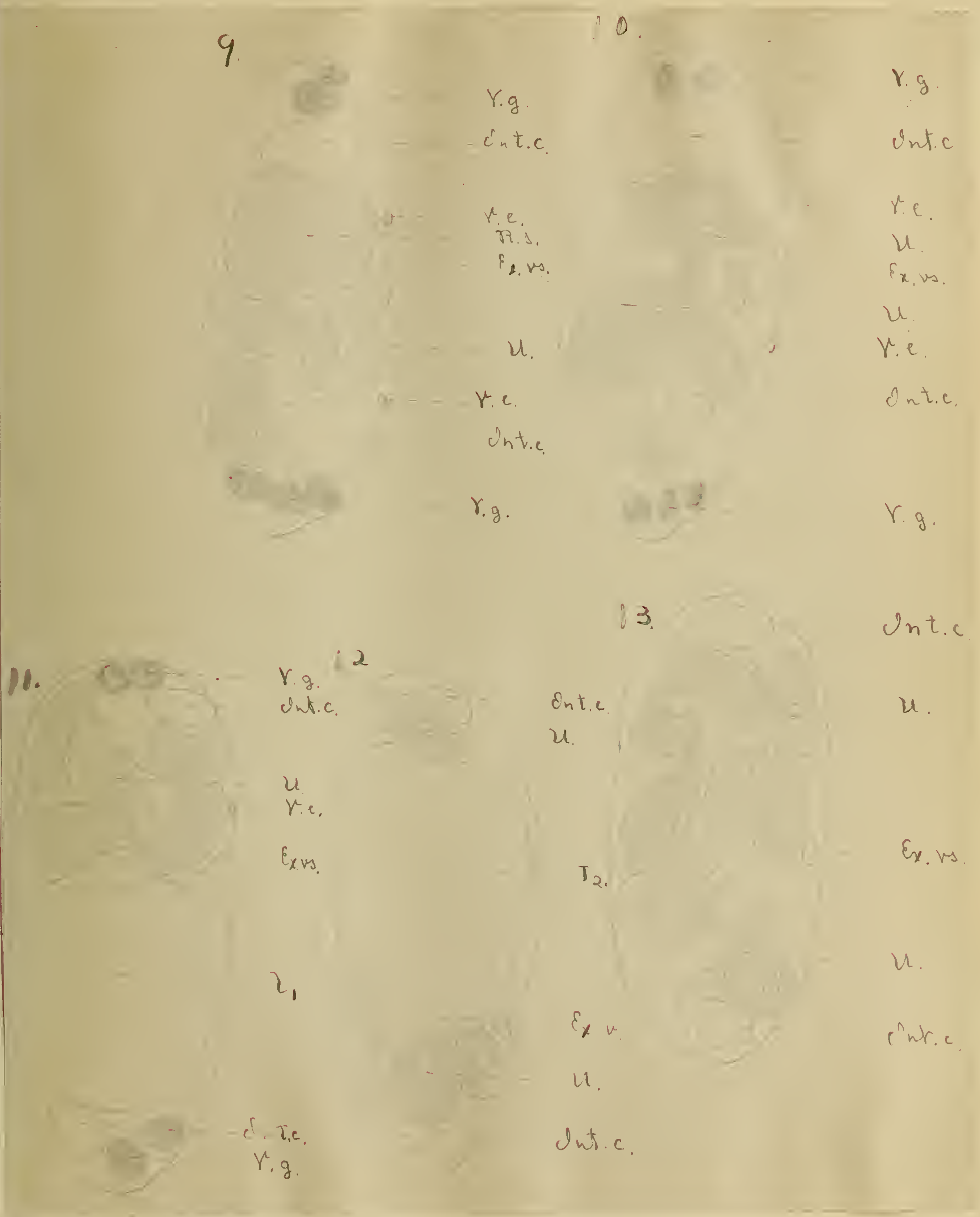

9
Y.g.
Int.c.
Y.e.
N.s.
Ex.vs.
U.
Y.e.
Int.c.
Y.g.
10
Y.g.
Int.c
Y.e.
U.
Ex.vs.
U.
Y.e.
Int.c.
Y.g.
13
Int.c
11.
Y.g.
Int.c.
U.
Y.e.
Ex.vs.
I_1
Int.c.
Y.g.
12
Int.c
U.
I_2
Ex v.
U.
Int.c.
U.
Ex.vs.
U.
Int.c

Fig. IV. b.

Connections of the Female Reproductive System in H. medioplexus. Ventral View. Diagrammatic.

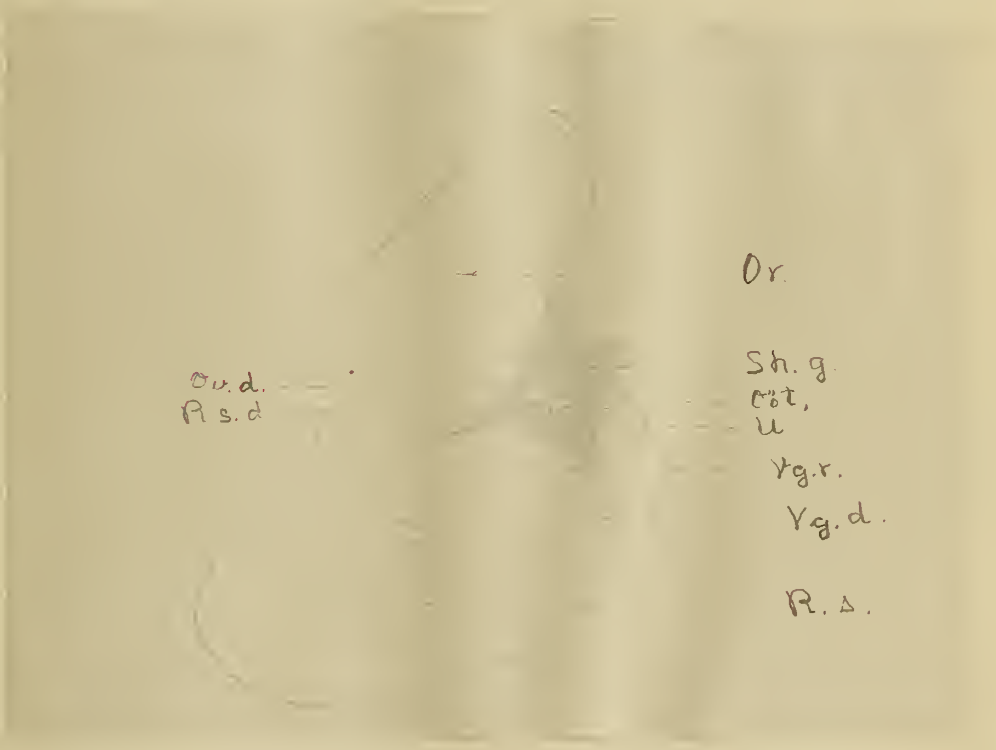


Fig. IV. c.

End Passages of the Reproductive Systems of H. medioplexus

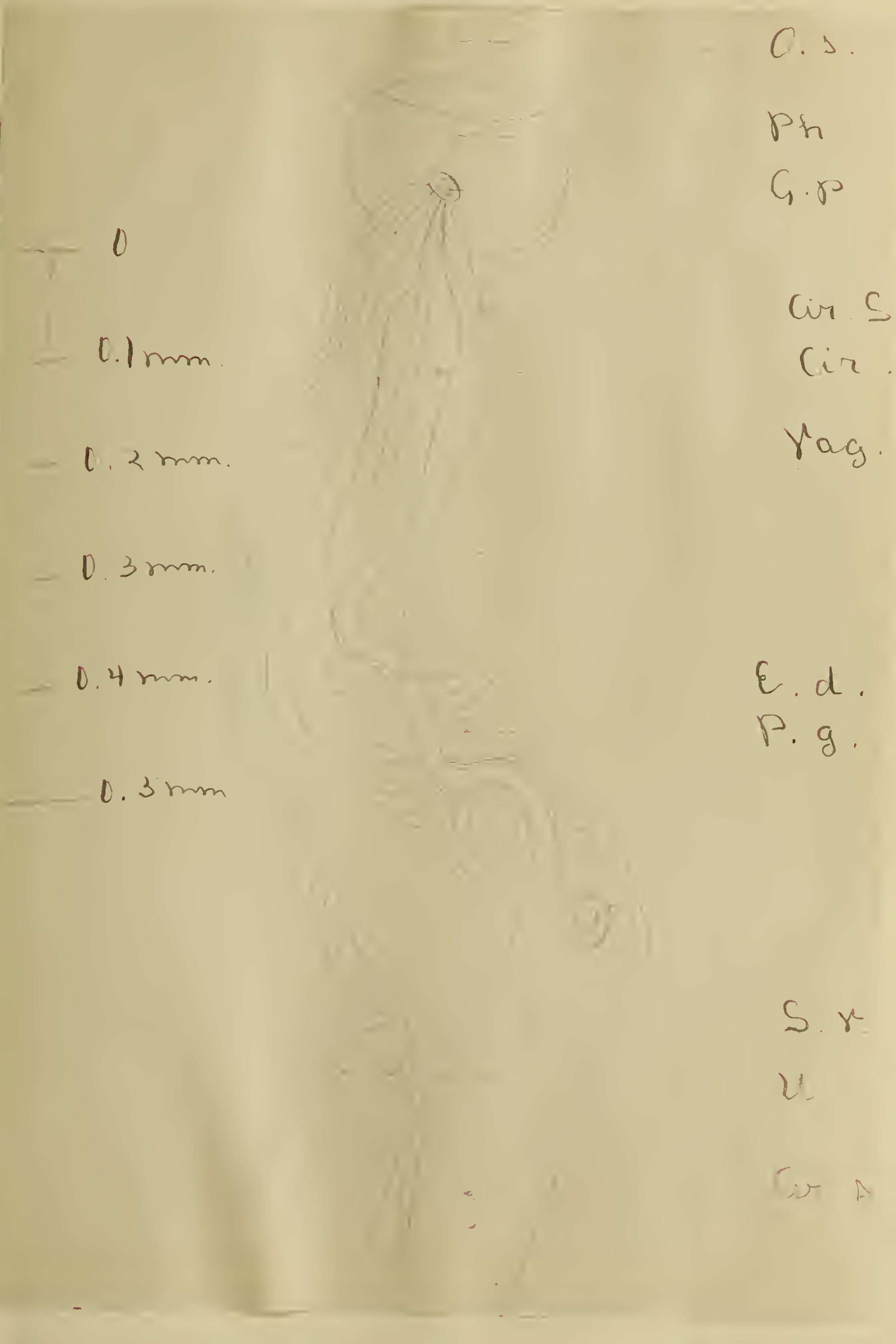


Fig. V.

Ostiolum formosum Pratt. Dorsal View.

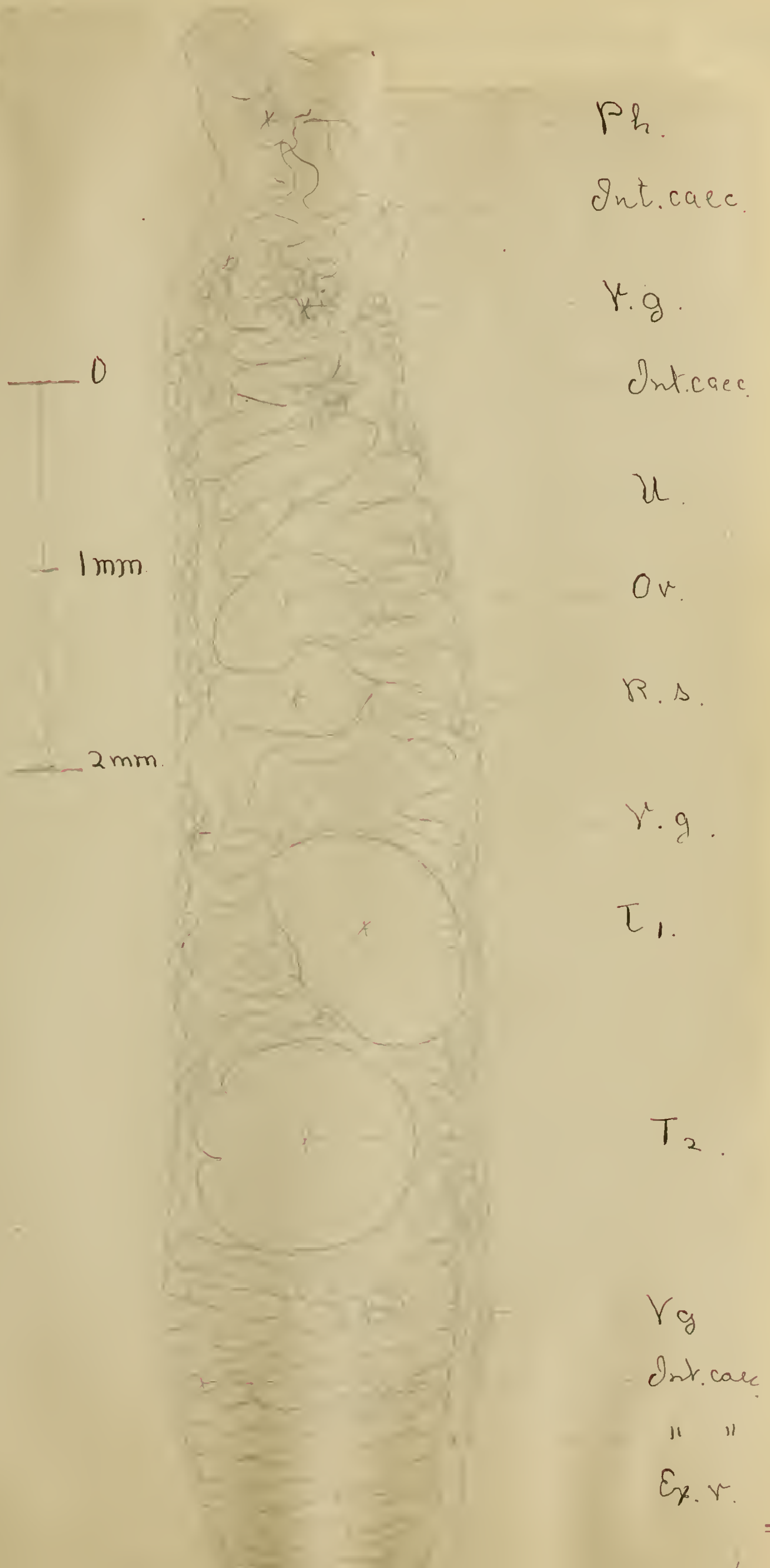


Fig. VI.

Posterior Region of a Specimen of H. medioplexus Showing Folds of the Uterus Outside the Intestinal Caesa. Dorsal View.

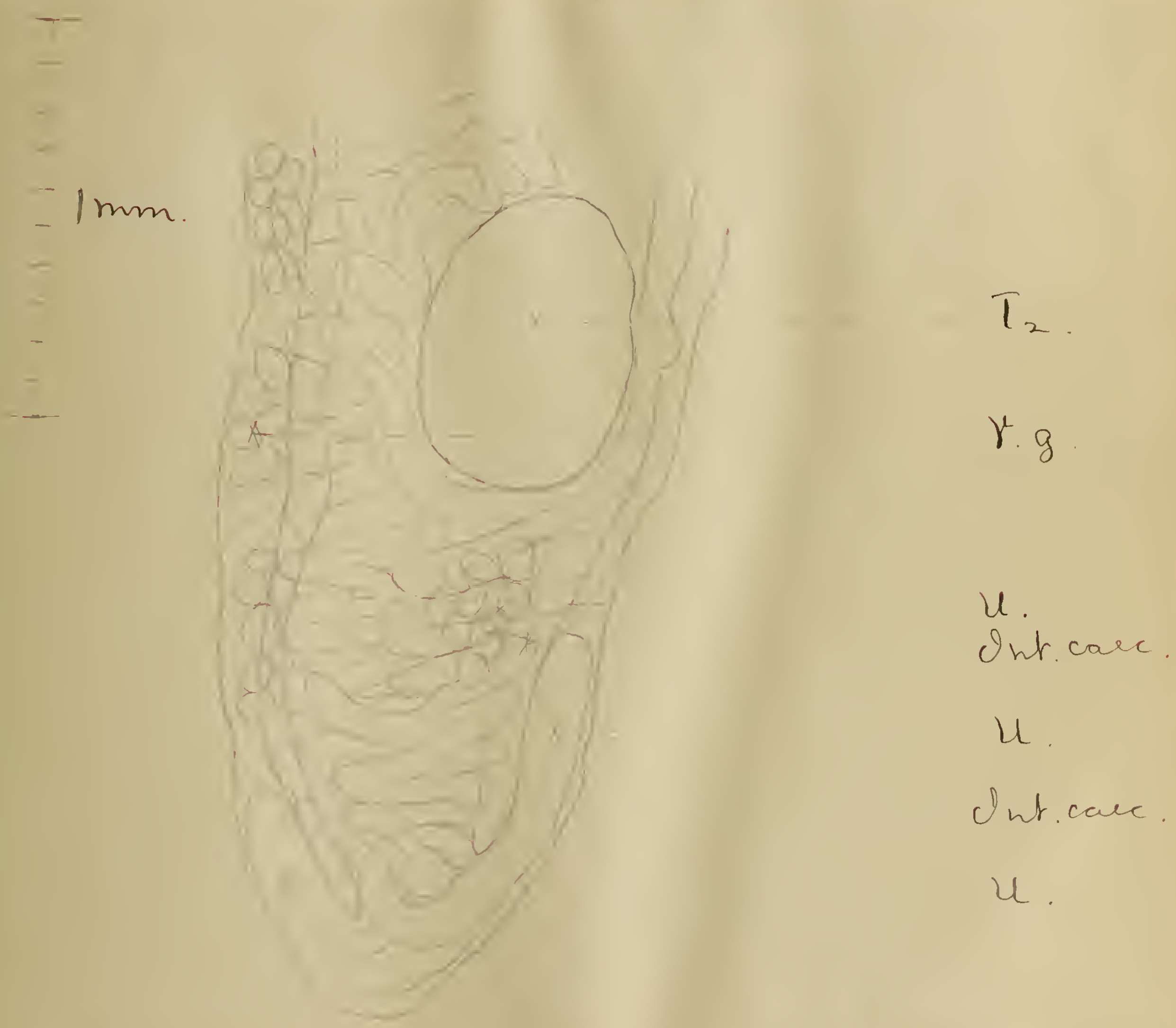


Fig. VII.

Living Specimens of H. longiplexus Showing the Amount of Contractility.

Fig. VIII.

Haematoloechus Eggs.

H. breviplexus

H. longiplexus

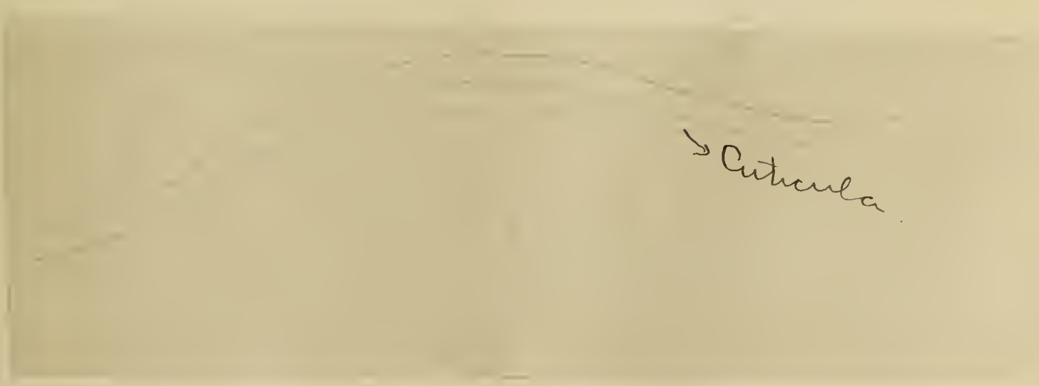
H. medioplexus

H. similiplexus

Fig. IX.

a. A Part of the Cuticula and Spines from H. medioplexus under high magnification.

b. From the Outer Edge of O. formosum Showing the Last Trace of the Sloughing Cuticula.



BLADDER FLUKES.

Zeder (1800:175) named the European frog bladder fluke *Distomum cygnoides*. Between that time and 1894 this form is mentioned a number of times and its life history partially worked out. Then Looss (1894: 56 - 64) describes this parasite in much detail and mentions the fact (p. 56) that two bladder flukes from the frog reported by older writers offer great variations. Rudolphi (Synops. 370) notes that von Gaede reports from Berlin in Bombinator igneus a bladder fluke 1 mm. long filled with eggs. Looss believes this to be an error of determination. He also considers that *D. vitellilobum* Olsson from the bladder of the frog, which has only two testes instead of nine as in *D. cygnoides*, is probably a young form of the latter species.

Bensley (1897: 326 - 331) describes two varieties of *D. cygnoides* from America, labeling them var. A & B. The first of these he notes as having nine testes and the second two.

Braun (1899:492) suggests grouping *D. folium*, *D. cygnoides*, *D. cymbiforme*, and *D. patellare* which occur in the bladder of cold blooded vertebrates into the genus *Phyllodistomum* with *D. folium* as the type species.

Later in the same year Looss (1899: 605 & 606) established the new genus *Gorgodera* for the frog bladder flukes with *G. cygnoides* as the type species, and included Bensley's *D. cygnoides var. A. & B.* under the names of *G. amplicava* and *G. simplex*. In his generic diagnosis he states that there may be either two or nine testes.

Stafford (1902: 411 - 424) re-described *G. amplicava* and

G. simplex, and added to this genus three new species from Canada, all of the two testes type, i.e. G. translucida, G. opaca, and G. attenuata. Toward the end of his description of G. amplicava, Stafford writes:

"The only point in which this species (G. amplicava) particularly resembles the European one (G. cygnoides Zeder) is in the possession of 9 testes, 4 on the one side and 5 on the other. So distinct a character is this that G. cygnoides and G. amplicava should be associated as a sub-group and separated from the rest - perhaps under a different generic name."

Looss (1902: 851) established for the form with two testes, the genus Gorgoderina, placing in it G. simplex and considering D. vitellilobum Olsson as a doubtful member.

Later Stafford (1905:687) accepted Looss' new genus and renamed his three species of 1902 Gorgoderina attenuata, Gn. opaca, and Gn. translucida.

Ssinitzin (1905: 33-39) re-described the old species of Olsson, and established it as a valid species Gorgoderina vitelliloba. In the same paper he describes a new species Gorgodera varsoviensis. He also points out the fact that the species described by Pagenstecher & Looss under the name D. cygnoides, are two distinct species and he renames them Gorgodera Pagenstecheri and G. Loossii, discarding entirely the specific name cygnoides. The validity of these names seems to me to be doubtful but at the present writing it is impossible for me to work out this point carefully.

At present two genera and nine species of frog bladder flukes are recognized as follows:

Gorgodera.

                                  (*G. Pagenstecheri* Ssin.
G. cygnoides Zeder (
                                  (*G. Loossi* Ssin.

G. amplicava Looss.

G. varsoviensis Ssin.

Gorgoderina.

Gn. simplex Looss.

Gn. vitelliloba Olsson.

Gn. attenuata Staf.

Gn. opaca. Staf.

Gn. translucida. Staf.

The European members of these genera have been described in much detail and their life history has been very thoroughly worked out by Ssinitzin (1905). The American forms, however, are very poorly known and there are no data on their life history. I have at my disposal at present material of *Gn. simplex*, *Gn. attenuata*, *G. amplicava* and a new species, *Gorgodera minima* n. s.

Gorgodera minima n. s.

Specific diagnosis.

Found in bladder of *Rana virescens*.

A very small, almost cylindrical worm, divided into two nearly equal parts by the acetabulum.

Ratio in size of the acetabulum to the oral sucker a little less than two to one.

Testes very close together, 5 on the same side as the

ovary and four on the opposite side, in shape rectangular prisms, with the depth about 3 times the thickness.

Seminal vesicle very large.

Vitellaria just back of the acetabulum in two groups of from 9 - 11 follicles in a group.

Ovary a solid body, rather irregular in shape and slightly lobed, having about the same general shape as the testes.

Uterus filling all the available space between the organs so that in old animals the posterior end of the body is a mere egg sac.

Eggs in alcoholic specimens from near the region of the genital pore 0.032 mm. by 0.022 mm.

In the fall of 1910 while examining for trematode parasites frogs from the region about the University of Illinois, I found in the urinary bladder of a large specimen of <u>Rana virescens</u> fifty very small trematodes which proved to be a new American species of the genus <u>Gorgodera</u>. They are closely related to <u>G. amplicava</u> Looss, which is the only other American representative of this genus, but they differ very markedly from this species in a number of particulars.

The wall of the bladder was thickly covered with these minute worms, - fifty in all. So tightly were they fastened by the oral sucker, which in some places seemed to penetrate the bladder wall, that much care was necessary in freeing them, and the tissue of the bladder was torn to shreds before they would loosen their hold. When free in the saline solution they showed much activity, extending and contracting very rapidly, the anterior end being the more contractile but the posterior end was by no means

sluggish.

When killed by the shaking method in corrosive acetic the worms became somewhat contracted, the living animals being about 1/3 longer than the preserved specimens. The preserved specimens also showed a tendency to bend at the acetabulum, forming often quite an angle. (Fig. II.) This makes this species difficult to mount and difficult to orient for sectioning. The ventral sucker divides the body of the animal into two almost equal parts, the anterior region being shorter and narrower than the posterior. Both these regions are almost cylindrical (Fig. IV, 1 - 5.); the posterior end, however, tapering slightly to a blunt point. Measurements from two specimens which were sectioned transversely bring out the relationship of these parts very clearly. The first of these is 0.9 mm. in length. In it the cross section at the middle of the anterior region measured 0.30 mm. in width by 0.26 mm. in thickness; at the region of the ovary which represents the widest part of the worm, the width was 0.30 mm. and the thickness 0.27 mm., and near the posterior end of the body at the region of the last testis, the animal was 0.24 mm. in width by 0.20 mm. in thickness. The above worm contained a number of eggs in the posterior end of the body, but none were present in the anterior folds of the uterus. The second specimen (Fig. IV, 1 - 5), in which every region of the uterus was crowded with eggs measured 1.4 mm. in length, In this worm the cross section thru the middle of the anterior end was 0.27 mm. in width by 0.26 mm. thick; at the region of the ovary 0.37 mm. in width by 0.31 mm. in thickness and thru the posterior testis 0.31 mm. in width by 0.27 mm. in thickness. These measurements show that both the anterior and

posterior regions in G. minima are nearly cylindrical.

In length my mounts vary from 0.9 mm. to 1.58 mm. In the smallest specimens a considerable number of eggs are present in the uterus of the posterior end, while in the longest ones the uterus thruout its length is crowded with eggs. In fact so abundant are the eggs in the form measuring 1.58 mm. that the internal organs are almost entirely obscured and the posterior end assumes the character of a distended egg sac. Besides the two specimens for which the widths were given above, two worms 0.95 mm. and 1 mm. in length measured in width 0.29 mm. and 0.32 mm. respectively. The width seems to bear no direct ratio to the length for in two toto mounts 0.95 mm. and 1.58 mm. the width for the first was 0.31 mm. and for the second 0.26 mm. The width varies, of course, directly with the state of contraction. In measurements of the suckers for ten specimens, I found the average size for the acetabulum to be 0.38 mm. in length by 0.39 mm. in breadth and for the oral sucker 0.19 mm. in length by 0.22 mm. in breadth, or a ratio of a little less than two to one. Stafford's description of the acetabulum (1902: 420) in G. amplicava as a bowl standing out from the body and attached by a narrowed base applies very well to G. minima.

The mouth appears in my preparations as a slit-like aperture toward the anterior part of the oral sucker, opening ventrally, and of about 1/5 or 1/6 the width of the sucker. From the mouth a short narrow oesophagus, about 0.017 mm. in width by 0.12 mm. in length, leads back without pharyngeal enlargement and divides into two intestinal caeca about 0.06 mm. in width. On account of contraction the forking in all my preparations is behind

the oral sucker, and the oesophagus could only be seen in sections. At the beginning of their course the intestinal caeca are dorsal, and wide apart to give room for the very greatly developed vesicula seminalis. (Fig. I.) In the narrow portion of the animal just dorsal to the acetabulum the caeca come closer together, (Fig. I) and just back of this organ they spread very widely to pass to the outside of the reproductive organs, which fill almost the entire width of the animal at this region. They continue backward outside of the reproductive organs and reach almost to the posterior extremity of the body. On one side of the animal the caecum lies next to the sidewall, while on the other, the proximal region of the uterus passing posteriad, runs to the outside of it.

In the relations of the male reproductive system G. minima agrees closely with G. amplicava and G. cygnoides. The differences are in the relative size of the parts, and in the distances between the different organs, which to some extent at least must be determined by the size and shape of the animal.

The testes are nine in number, five on the same side as the ovary and four on the opposite side. They are in shape rectangular prisms, crowded very closely together in my preparations. The average measurements for a single testis are 0.05 mm. with the length of the worm, 0.08 mm. with its width and 0.24 mm. dorsoventrally. This is a very interesting condition, and an important distinguishing feature of the species. One might compare them to two series of cigar boxes attached by strings thru their middles and arranged four on one side and five on the other with the string from each side series connecting farther forward. The testes them-

selves are somewhat irregular in outline, slightly lobed, and connected by a series of very short tubules. From the middle of the anterior surface of each anterior testis runs forward a vas efferens. These tubules unite in front of the ovary and yolk glands into the vas deferens, which ends in the vesicula seminalis. This organ in my specimens is a large pyriform sac, folling a slightly spiral course, and filled with sperms. In a worm 0.9 mm. long it measured 0.14 mm. in length, and in a cross section 0.30 mm. in width by 0.26 mm. in thickness it measured 0.09 mm. in width by 0.11 mm. in thickness. In an older animal 1.4 mm. long, the seminal vesicle was 0.18 mm. long and measured 0.18 mm. in width by 0.21 mm. in thickness. Of course these measurements are somewhat modified by the state of contraction of the worm, but I give them to show the relative large size of the seminal vesicle of this species. From the anterior end of the seminal vesicle the ejaculatory duct curves over the front of this organ, and opens into the common genital pore.(Fig.IV) This duct widens out before opening into the pore into a small chamber lined with epithelial cells, among which are heavily staining club-shaped cells, which appear to be glands. Around the ejaculatory duct are grouped the prostate glands. (Fig.V.)

The vitellaria (Fig. I.) in G. minima are composed of two groups of 9 - 11 follicles each, lying one to each side of the animal and just back of the acetabulum. They are connected by a transverse vitelline duct which empties into a common vitelline duct. Immediately behind these organs and to the left side is the ovary, which is an irregular slightly lobed very deep organ, in depth almost equalling the thickness of the animal. In a cross section thru the middle of the ovary - 0.28 mm. wide by 0.26 mm. thick, - this organ was 0.12 mm. wide by 0.20 mm. thick. It lies nearest the ventral surface and is widest toward this side. In a frontal section 1.1 mm. long by 0.28 mm. wide the ovary measured

0.1 mm. with the length of the animal and 0.15 mm. in width. None of my specimens showed sexual amphitypy. In twelve, which I examined the ovary was to the left. The oviduct leaves the dorsal surface of the ovary, to widen into the fertilization space, and pass forward still near the dorsal surface into the "shell" gland. This is a small group of unicellular glands which lies toward the dorsal side of the animal between the groups of follicles of the vitelline glands. Within the shell gland the oviduct receives the median duct from the yolk glands. Laurer's canal is present opening into the oviduct just in front of the fertilization space. From its junction with the oviduct this duct passes ventralward and then doubles back to open on the dorsal surface of the animal just back of the ovary. As with the testes, the longest axes of the vitellaria and ovary are with the thickness of the animal. This fact which is of course correlated with the great dorso-ventral thickness in this species, is an important diagnostic feature.

The uterus takes its origin from the oötype, passes to the side opposite the ovary and folds down the region outside the testes on that side to the posterior extremity of the body, where it fills with its coils the region back of the ends of the intestinal caeca and testes. From here it winds forward on the opposite side of the body, filling not only the region between the groups of testes on each side, but also all the available space between the testes and the body walls, and even between the individual testes. In front of the testes it emerges from this mass of coils, to pass to the ventral side of the ovary, over the acetabulum and forward ventral to the vesicula seminalis to the genital pore. In such a

uterus as the one described above the whole course is distended with eggs. In younger forms where fewer eggs are present there is less complication. The general course then of the uterus, as described above, is down the side opposite the ovary and up the other side to the genital pore filling all the available space between the organs.

The eggs in my species, as in Gorgodera and Gorgoderina, in general increase in size as they develop in the uterus from the oötype to the genital pore. In preserved specimens of G. minima near the oötype the eggs average in size about 0.021 mm. x 0.014 mm.; in the coils of the posterior end 0.024 mm. x 0.017 mm., and just below the genital pore where they contain well developed embryos 0.032 mm. by 0.022 mm. Looss (1899:63) notes similar differences in size for the eggs of Distomum cygnoides. I have no measurements of the eggs from living specimens. As noted by Stafford and observed by me in closely related forms, the eggs in this group shrink greatly in animals which have been préserved in alcohol. Therefore, the only trustworthy measurements of eggs for comparison would be of those taken from live animals.

G. minima is the second American species of the genus Gorgodera, the other species being Gorgodera amplicava, described by Bensley. The most striking differences between these two species are in the size and shape of the animals and ratio of the suckers. G. minima is a very small worm 1 - 2 mm. in length, with the anterior and posterior regions of almost equal size and almost cylindrical. G. amplicava on the other hand is much larger (3 mm. - 5 mm. in length), and also much wider, with the posterior end

pointed and flattened like the blade of a wide two-edged knife. The anterior region is very small in proportion to the posterior and cylindrical, being about 1/3 as long as the posterior region and not ¼ as wide altho thicker. In *G. amplicava* the acetabulum is not only twice as large as in G. minima, but is from 2½ - 3 times the size of the oral sucker, while in the latter species the ratio is less than 2 : 1. The great relative thickness of the testes and ovary in *G. minima* is another feature which differentiates it from *G. amplicava*. The seminal vesicle is much larger relatively in the former than in the latter species. The European species of this genus are much larger than the American forms, all being over 7 mm. in length. The new species is by far the smallest representative of the genus Gorgodera.

Gorgoderina attenuata Stafford

Stafford (1902: 418-419) describes Gorgoderina attenuata from the Canadian frogs, Rana virescens and R. catesbiana. I have found this form in R. pipiens, and have besides specimens from R. catesbiana from Rice Lake, Ontario.

In thirty R. pipiens examined thirty-seven of these flukes were found in nine hosts, the heaviest infection in a single frog being nine. The specimens from R. catesbiana are a little smaller and more attenuated than the average of those from R. pipiens. This species is easily recognized on account of the extreme attenuation of the body, and the relatively large size of the acetabulum.

These flukes show considerable power of movement. With the acetabulum attached firmly to the wall of the bladder, the posterior end would often be stretched out to two or three times its normal length, become very slender, and lash violently from side to side. When loosened they showed considerable movement, stretching both the anterior and posterior ends actively. The total length of these worms is about 10 - 12 times the width. The ventral sucker is like a very large bowl attached at its base and dividing the animal into two distinct regions. Dorsal to it there is only room for the intestinal caeca and for the ducts of the different systems to pass up to the anterior end. (Fig. VII, 2.) The anterior region is cylindrical and narrow, comprising about 1/3 - 1/4 the length of the animal. The region posterior to the acetabulum is over $\frac{3}{4}$ (Fig. VII, 3, 4, 5.) as thick as wide, and ends in a rather sharp point.

Sixteen of the specimens from Rana pipiens varied from

5.3 mm. to 7.2 mm. in length. The Canadian material is slightly smaller ranging from 3.5 - 4.8 mm. The width depends somewhat on the state of contraction varying from about 0.35 mm. to 0.56 mm. Measurements of the cross sections of three different flukes in the widest region gave 0.46 in width by 0.35 in thickness, 0.56 in width by 0.46 in thickness, and 0.54 in width in width by 0.46 in thickness.

The oral sucker in 16 specimens ranged from 0.26 - 0.33 mm. in diameter, and the acetabulum (Fig. VI & VII, 2.) from 0.6 mm. - 0.82 mm. The ratio varied from 1 : 2.1 up to 1 : 3, with an average of 1 : 2.5.

The digestive system offers few points of importance. The mouth opens ventrally thru the oral sucker into the narrow oesophagus about 0.4 mm. long. This divides into the intestinal caeca (Fig. VII, 1-5.), which pass backward wide apart in the region occupied by the reproductive organs, but back of the posterior testis they approach each other, and terminate almost in contact not far from the posterior extremity of the animal.

The testes (Fig. VI & VII, 4.) are large, rather rounded slightly elongated structures lying diagonally on opposite sides of the body, with the posterior on the same side as the ovary. They are thicker than wide and lie toward the dorsal surface. In a mount 5.2 mm. in length the anterior testis measured 0.78 mm. in length by 0.30 mm. in width and the posterior 0.93 mm. in length by 0.32 mm. in width. In a cross section 0.47 mm. in width by 0.44 mm. in thickness (Fig. VII, 4.) the testis measured 0.20 mm. in width by 0.26 mm. in thickness. From the anterior end of each testis the

vas efferens passes forward. They keep a dorsal course and unite just in front of the vitellaria to form the vas deferens. Just in front of the attachment of the acetabulum the vas deferens enlarges into a large seminal vesicle, which is a large pyriform sac filled with sperms. (Fig. VII, 1 & VIII.) From the dorsal margin of the anterior end of this organ, the ductus ejaculatorius (Fig. VIII) goes directly ventralward to the genital pore, having clustered along the middle of its course the prostate glands. There is no enlargement of the end of this duct as in Gorgodera minima. (Fig. V.)

The ovary (Fig. VI, VII, 3, & IX.) is a small round or oval body lying just in front and to the opposite side of the body from the anterior testis. In seven out of thirteen specimens the ovary was to the right. In the same preparation for which the measurements of the testes are noted above, the ovary measured 0.32 mm. in length by 0.24 mm. in width. It is wider than thick and lies toward the ventral surface. In a cross section 0.31 mm. in width by 0.33 mm. in thickness (Fig. VII, 3,), the ovary measured 0.22 mm. in width by 0.20 mm. in thickness.

The connections of the female reproductive system (Fig. IX.) are toward the dorsal surface. The oviduct passes out from the mid dorsal part of the ovary and widens almost immediately into the fertilization space. It becomes narrower again and is joined by Laurer's canal before entering the "shell gland." Within the "shell gland" it receives the short median duct from the yolk gland. The beginning of the uterus passes forward, curves over between the yolk glands, and is lost in a mass of coils which fill the posterior region.

The vitellaria (Fig. IX.) are compact organs, lobed, but

not divided into follicles. There is one gland on each side and they are connected by a transverse duct.

The coils and folds of the uterus in the posterior region of Gn. attenuata are so numerous that it is impossible to distinguish any definite arrangement. The whole body back of the acetabulum is so crowded with eggs that all the organs are more or less obscured. In front of the vitellaria the uterus makes several voluminous transverse folds (Fig. VI.) and then passes forward along the mid line of the body ventral to the vas deferens up to the genital pore. The last part is modified into a short vagina.

Eggs from living specimens near the genital pore measured about 0.053 mm. by 0.034 mm. Eggs from the same region in alcoholic specimens measured about 0.032 mm. by 0.022 mm. In the largest living eggs miracidia can be seen moving around fully developed. Such eggs, when placed in distilled water, began to pop open within 10 or 15 minutes, liberating the miracidia. These larvae, which are cylindrical and pointed at one end swarmed very rapidly across the field of the microscope, swimming with a rapid whirling motion. Gradually they became distorted and slowed up.

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Abbreviations Used in Figures.

Ant. reg. - Anterior region.

Ac. - Acetabulum.

E. - Eggs.

Ej. d. - Ejaculatory duct.

F. s. - Fertilization space.

G. p. - Genital pore.

Int. c. - Intestinal caeca.

L. c. - Laurer's canal.

O. s. - Oral sucker.

Oes. - Oesophagus.

Oöt. - Oötype.

Ov. - Ovary.

Post. Reg. - Posterior region.

P. g. - Prostate glands.

S. v. - Seminal vesicle.

Sh. g. - Shell gland.

T. - Testis.

V. g. - Vitelline gland.

V. g. d. - Duct of vitelline gland.

V. d. - Vas deferens.

Vag. - Vagina.

Fig. I.

Gorgodera minima n. s.

Ventral view. Toto mount.

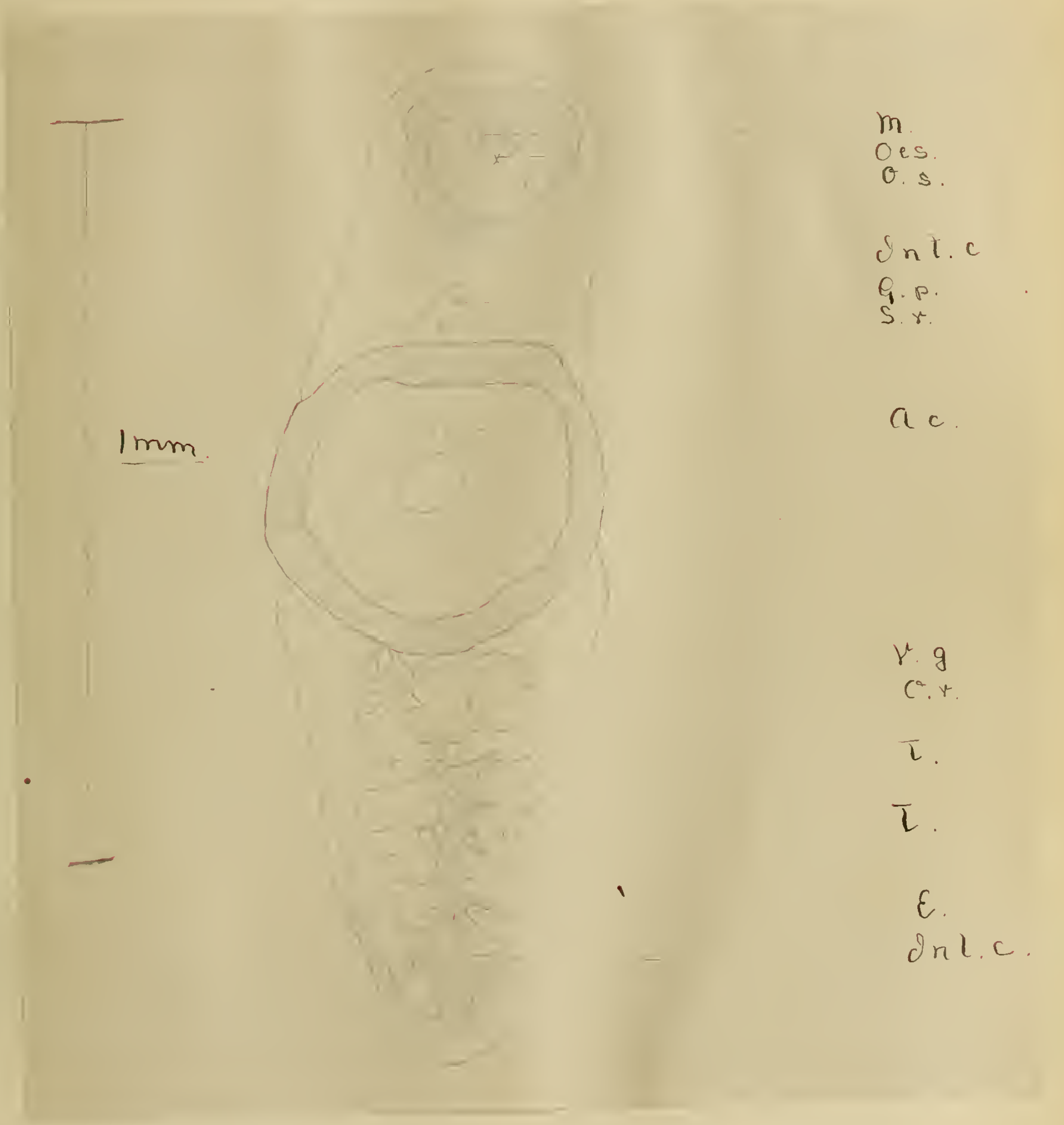

Fig. II.

Gorgodera minima

Side View to Show the Relation of the Acetabulum to the anterior and posterior regions of the animal.

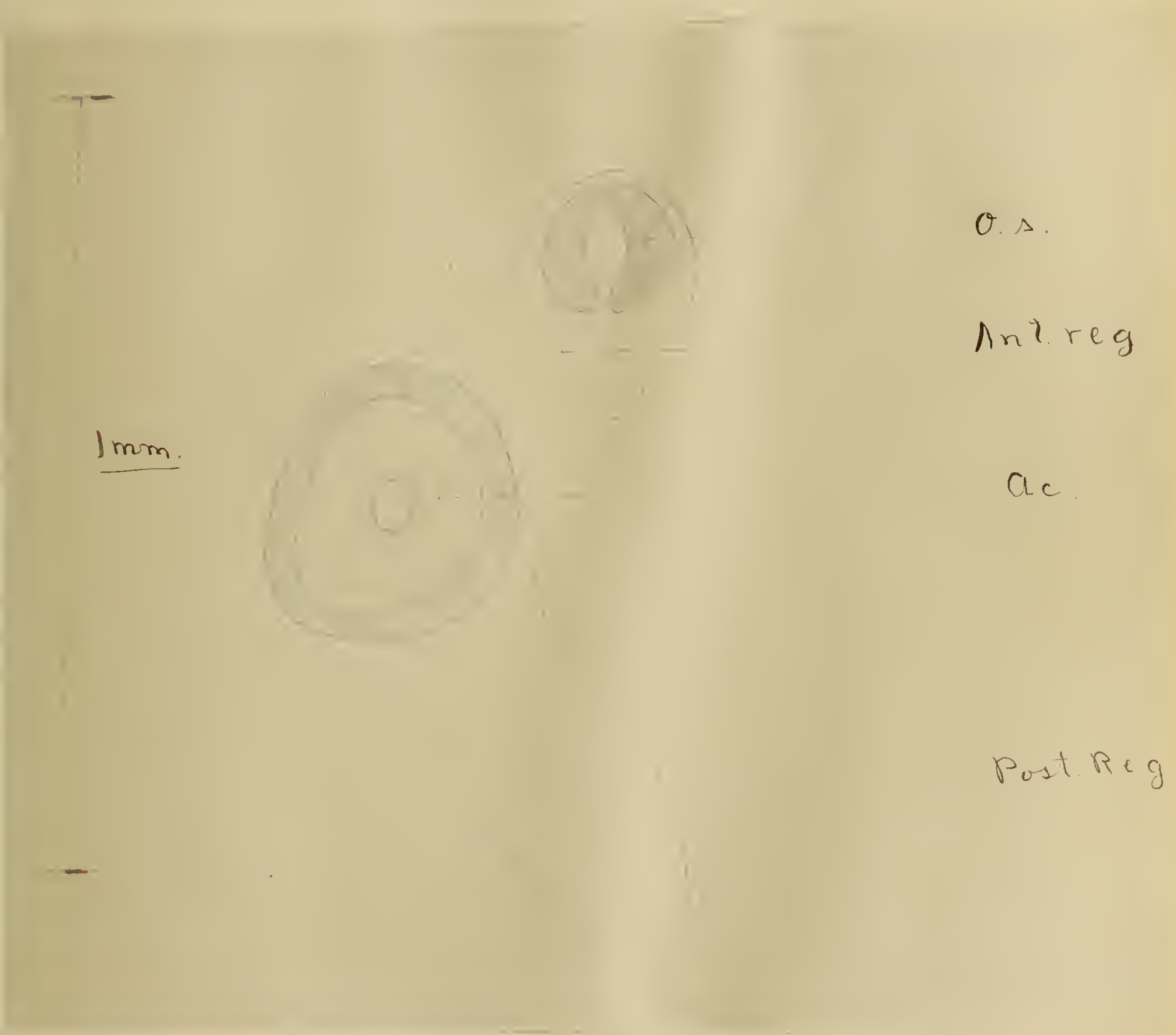

Fig. III.

Suckers. G. minima

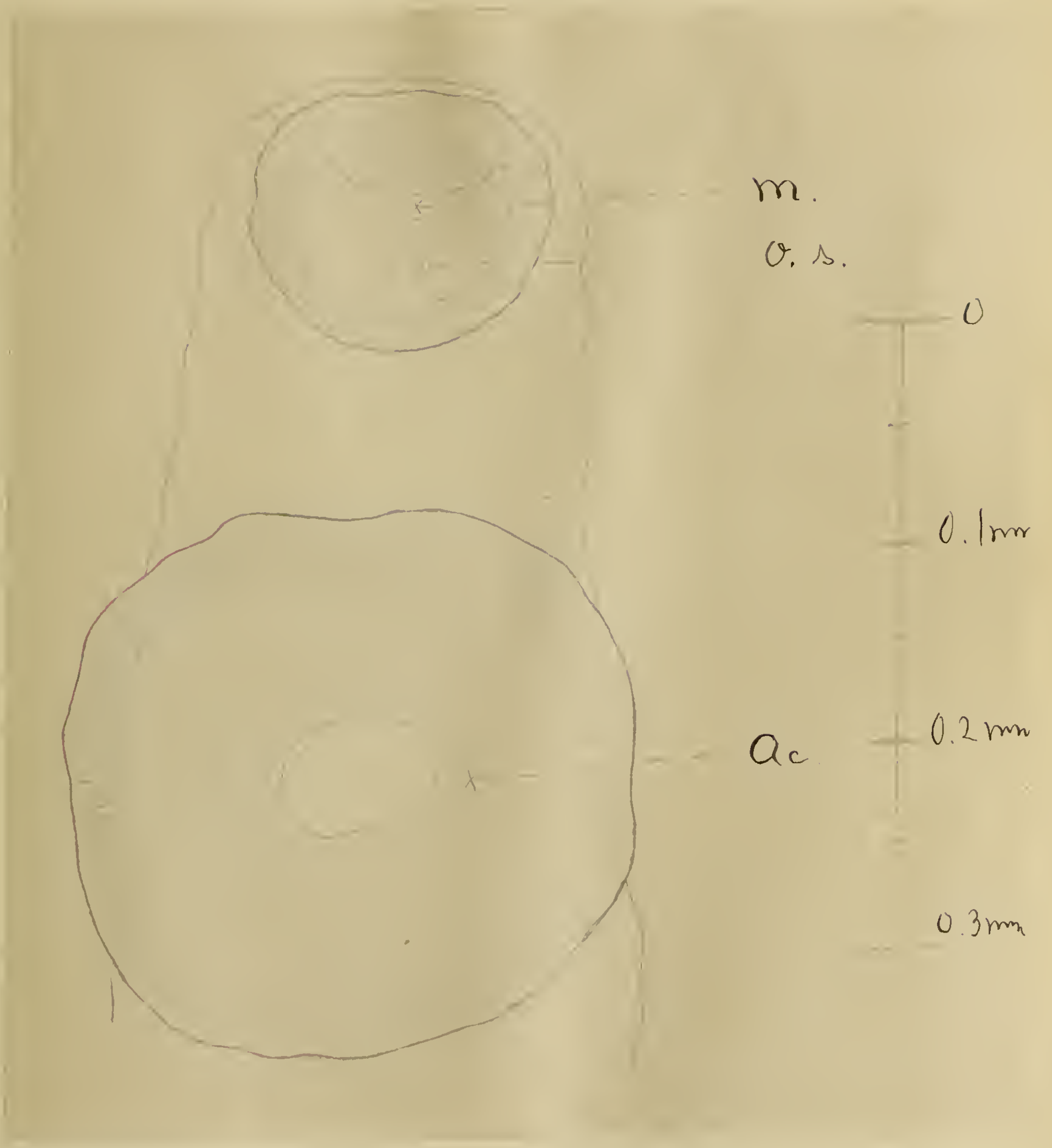

Fig. IV.

Series of Cross Sections of *G. minima*.

Ventral Side Down.

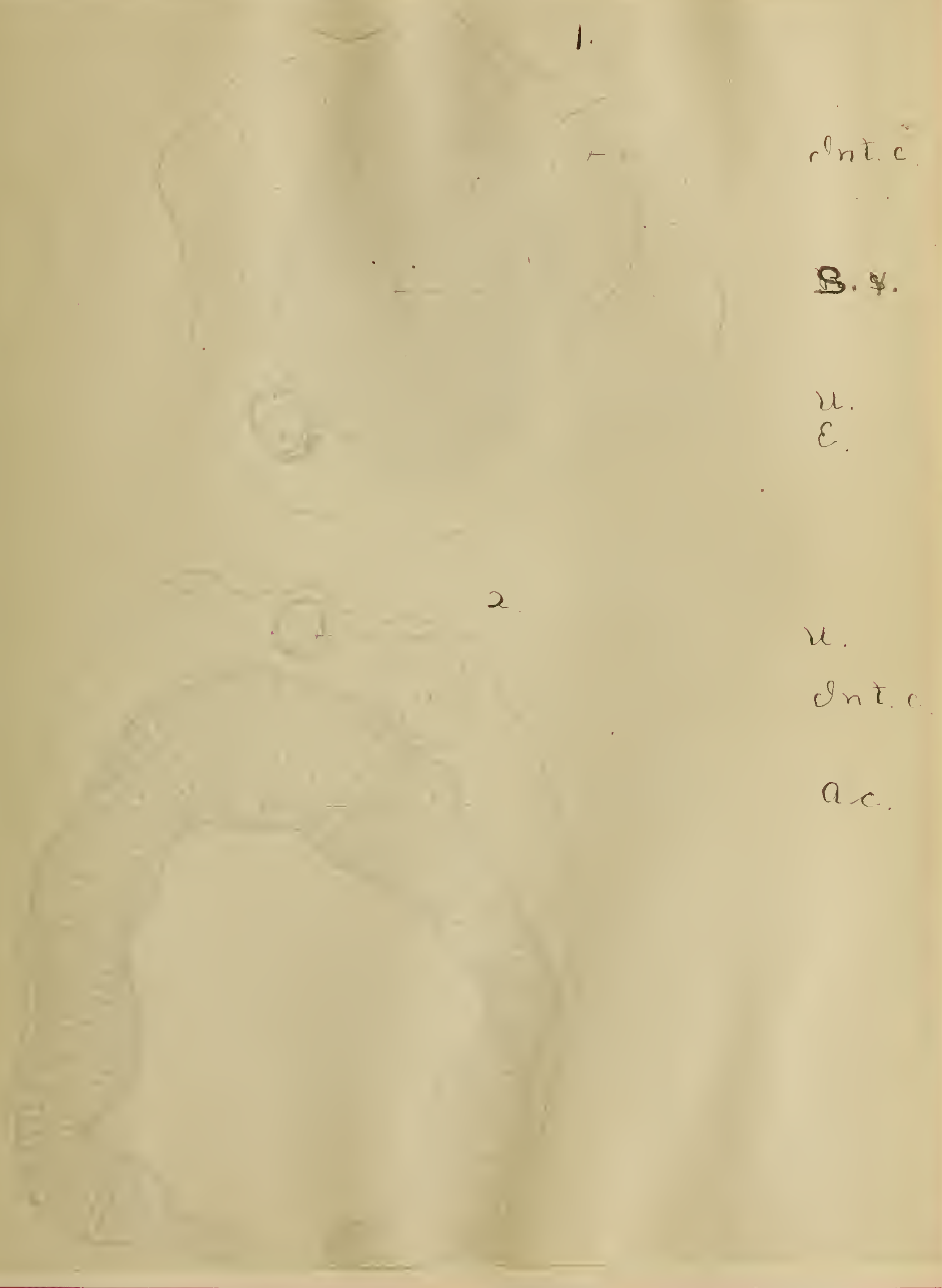

Fig. IV. (cont.)

3.

4.

Fig. IV. (cont.)

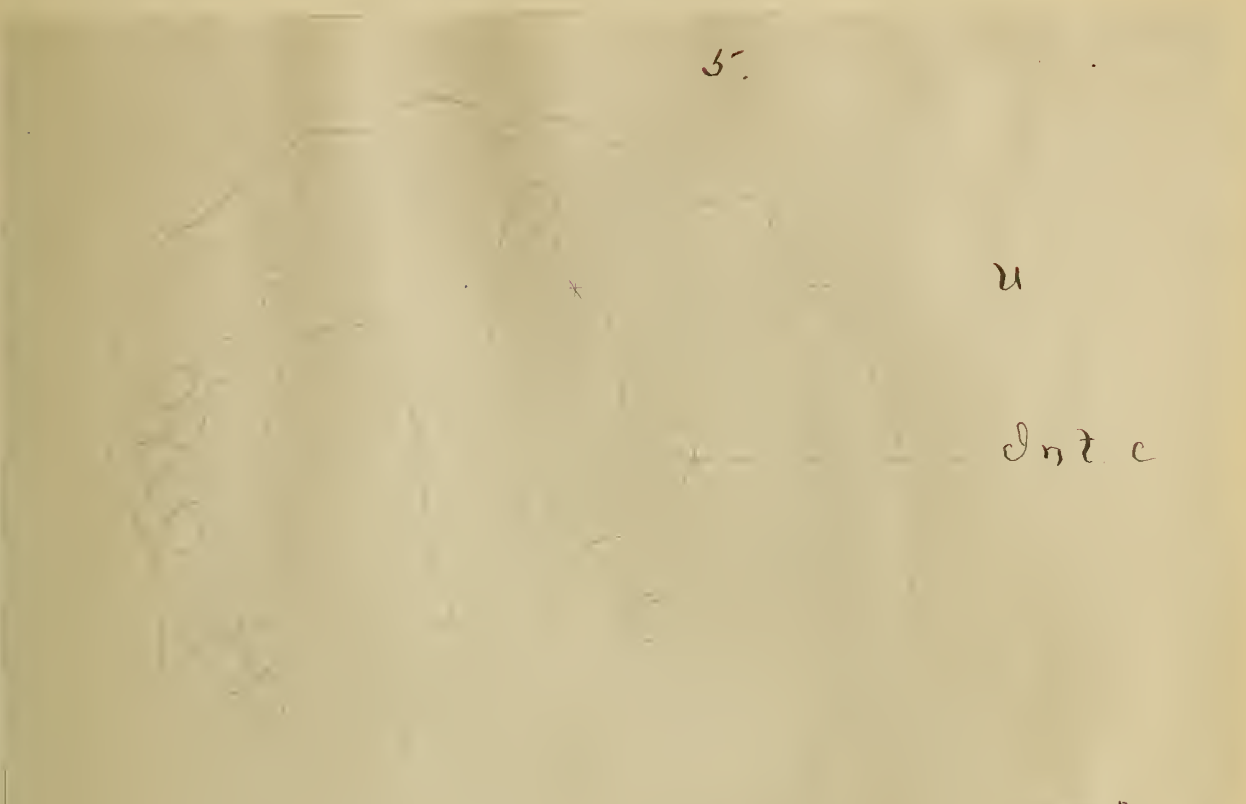

Fig. V.

End Ducts of Reproductive Systems.

G. minima. Reconstruction Sagittal. Sect.

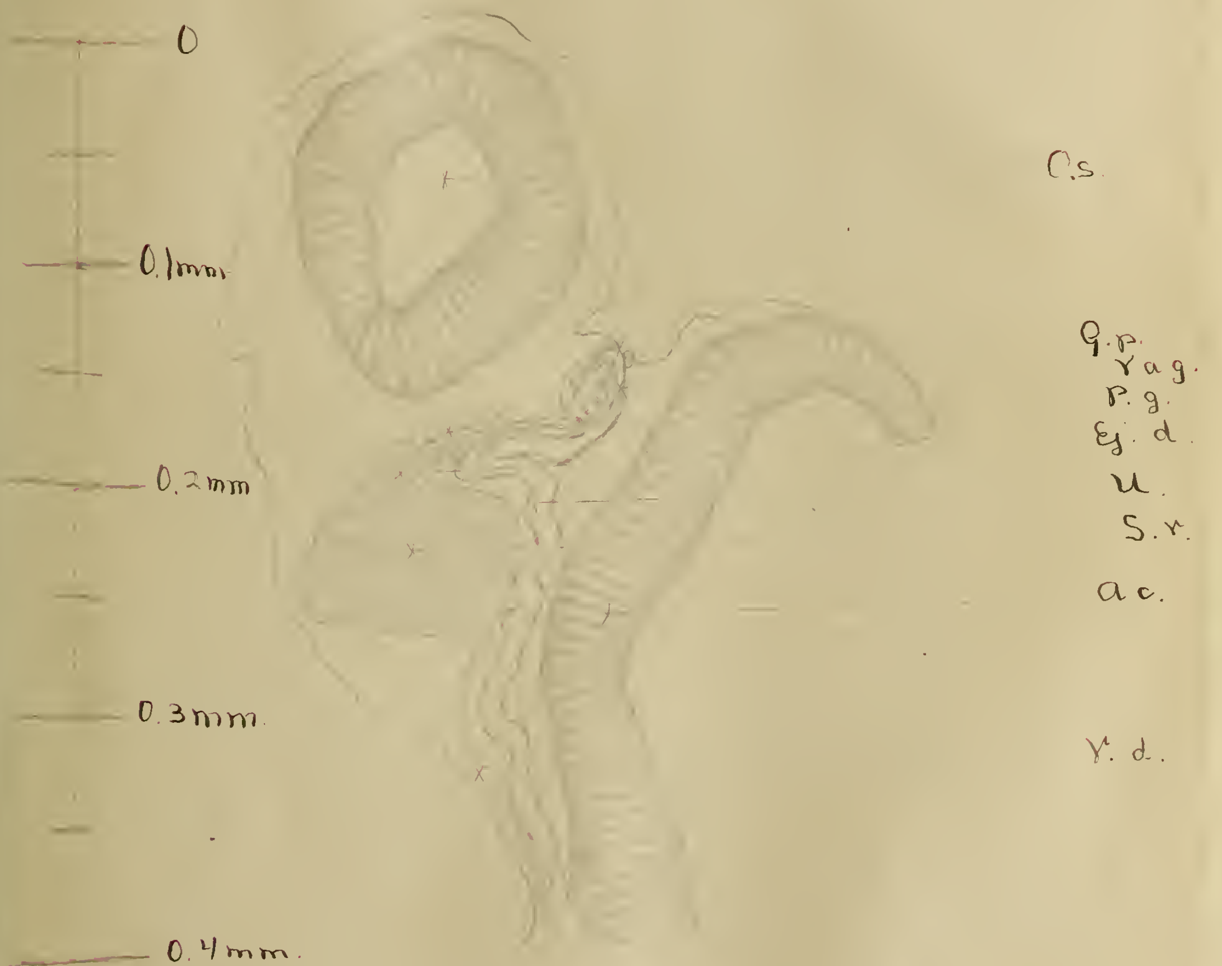

Fig. VI.

Gorgoderina attenuata.

Dorsal View. Toto

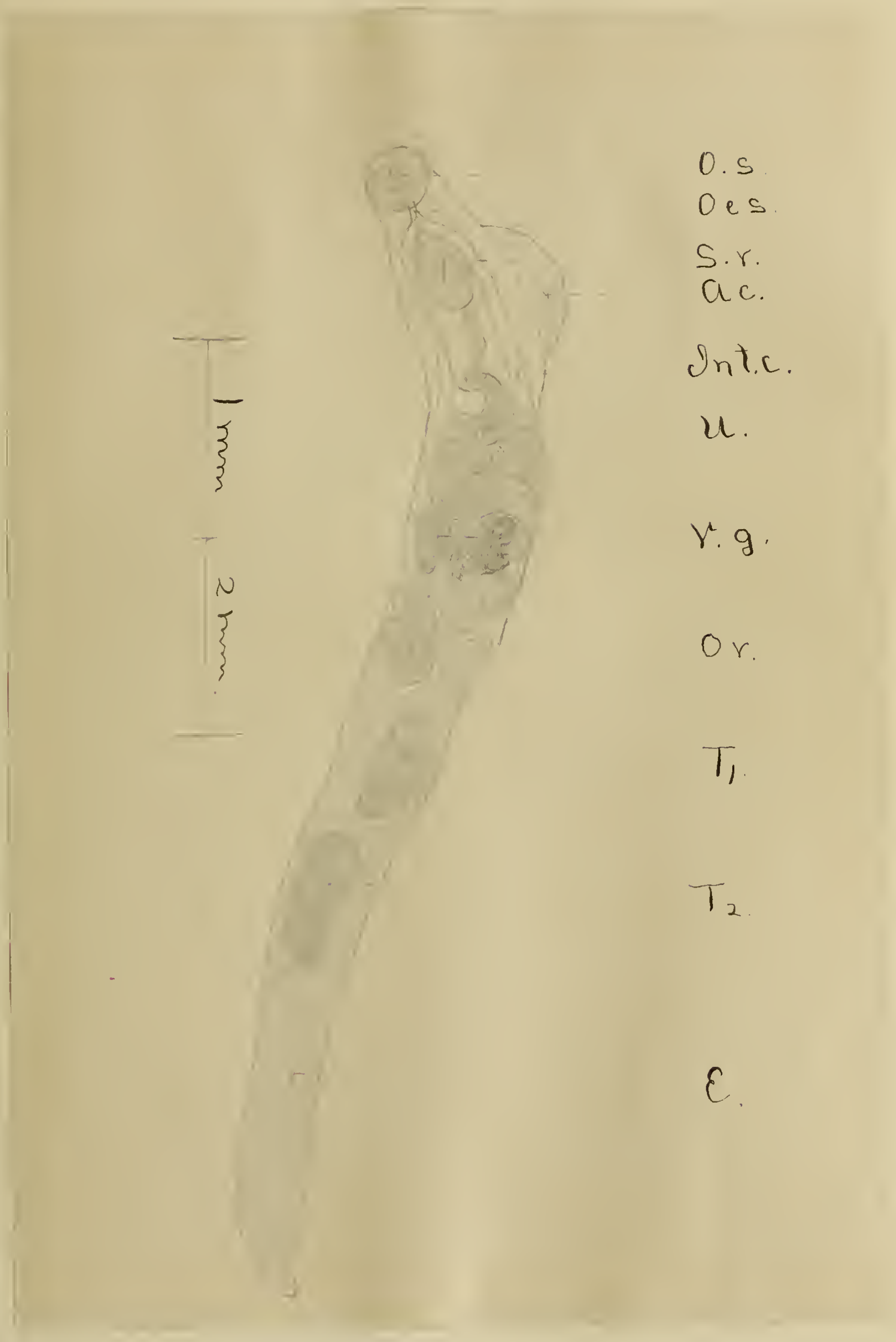

Fig. VII.

Cross Sections. Gr. attenuata.

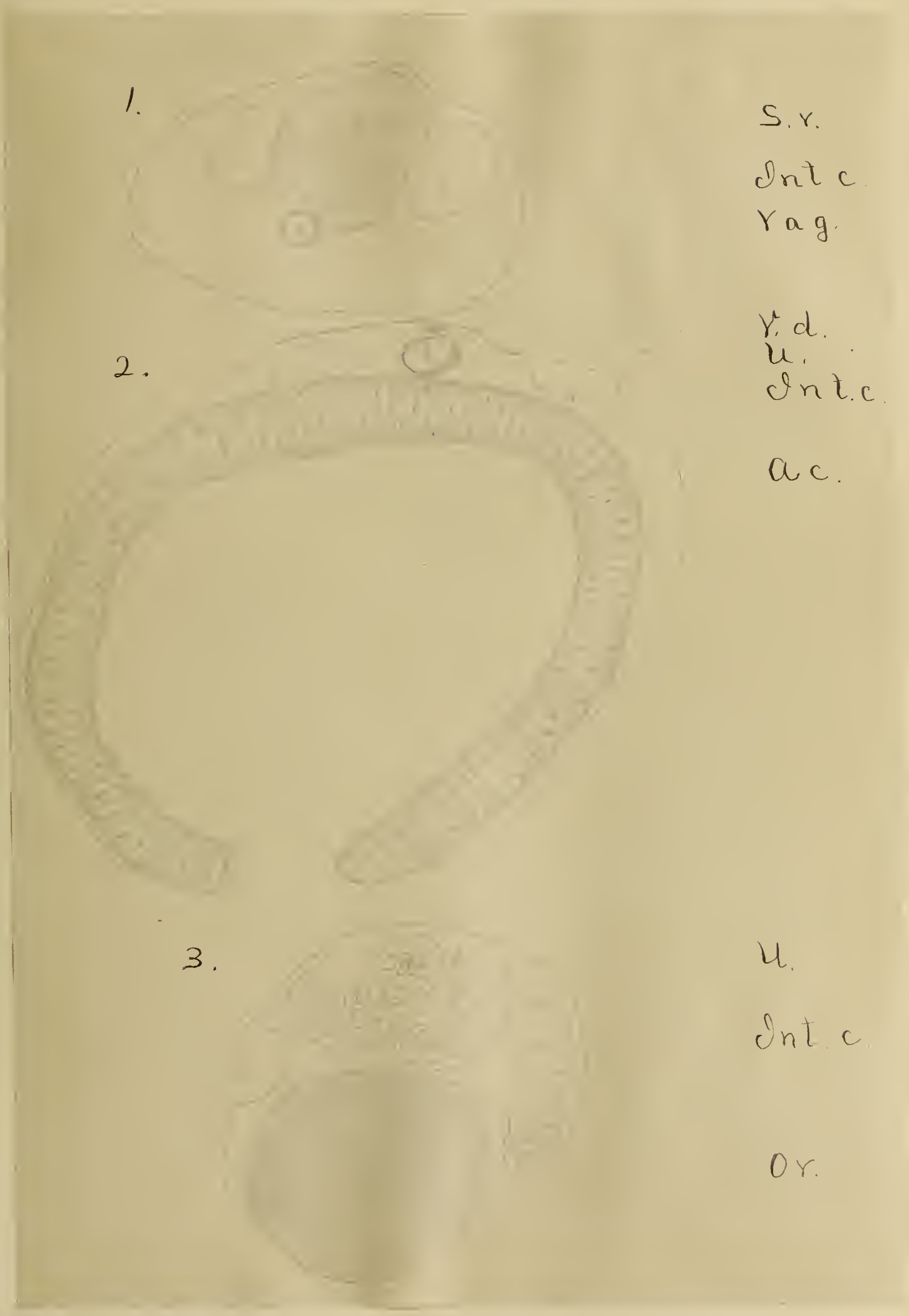

Fig. VII. (cont.)

Cross Sections. Gn. attenuata.

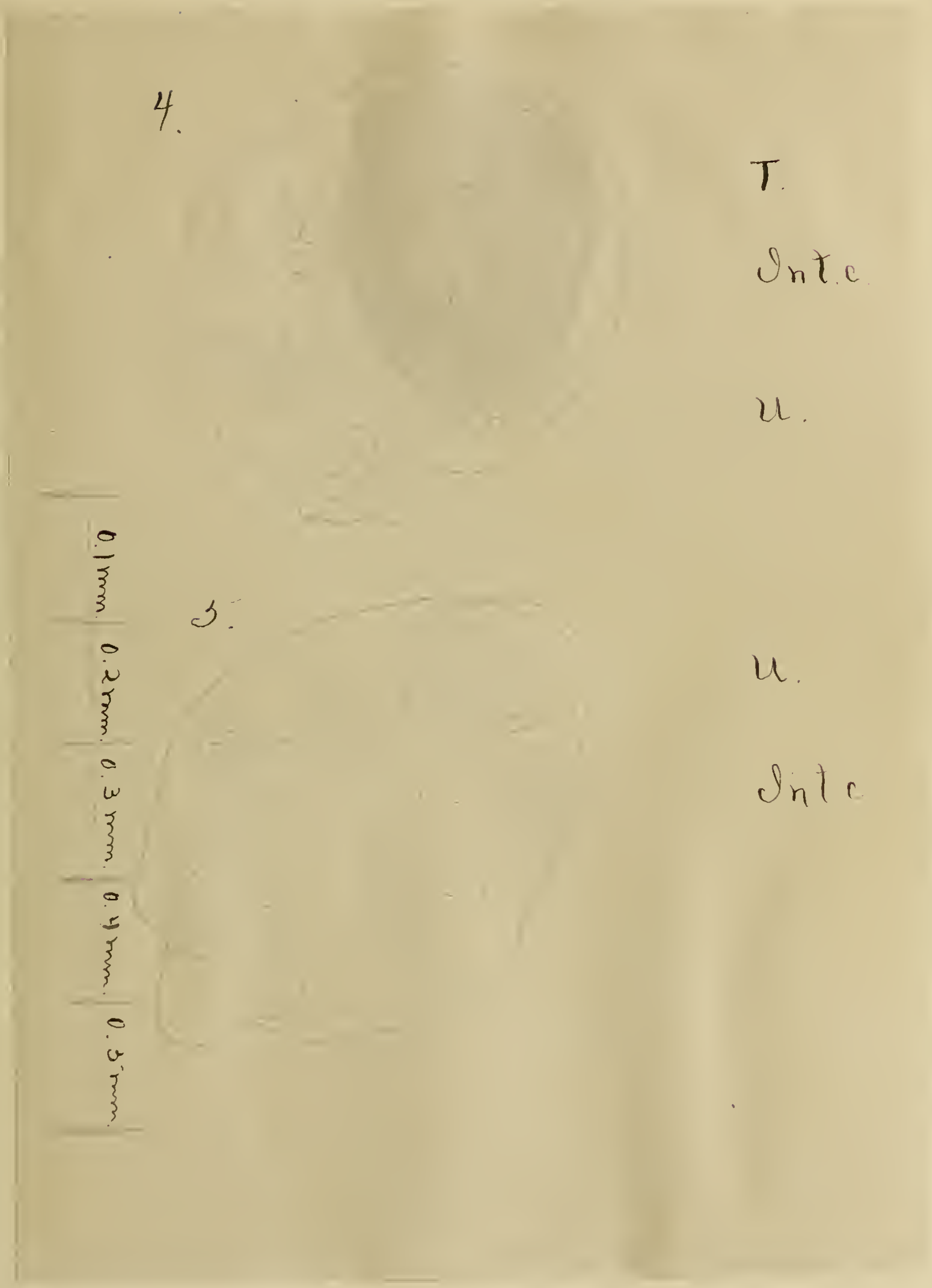

Fig. VIII.

Endings. Reproductive Systems. Gn. attenuata.

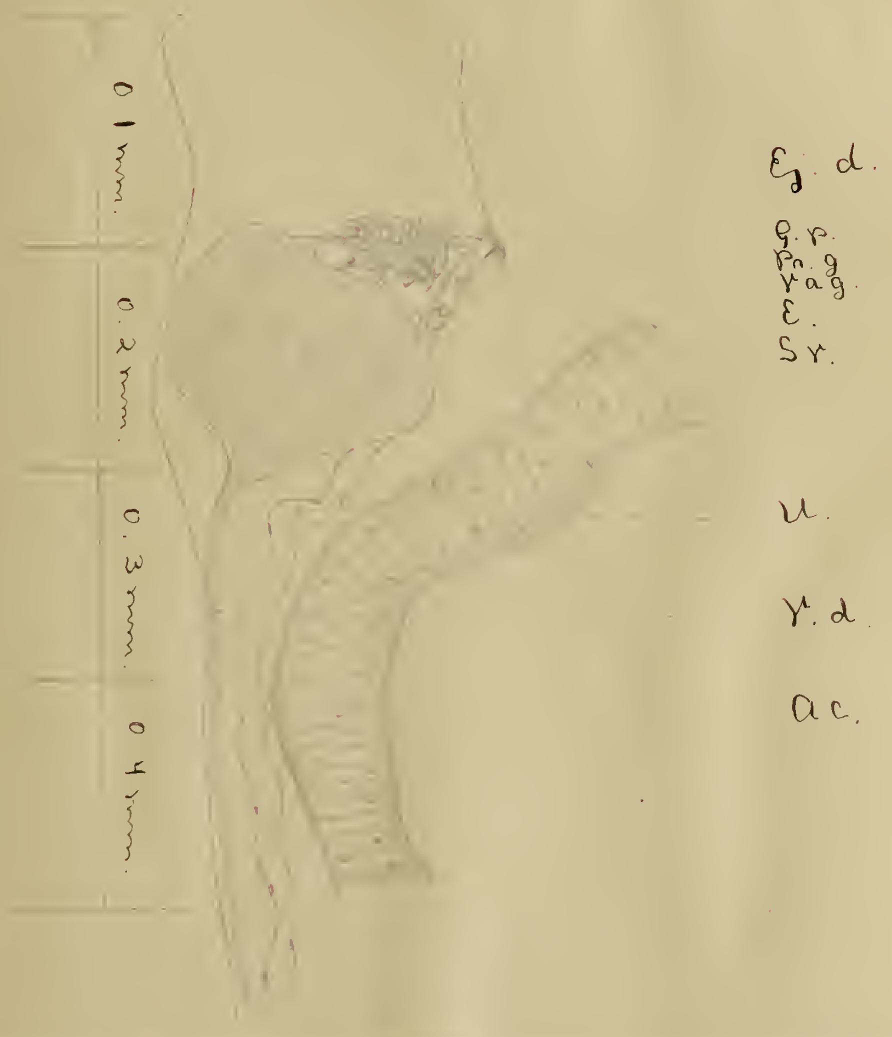

Fig. IX.

Connections. Female Reproductive System. Gn. attenuata.